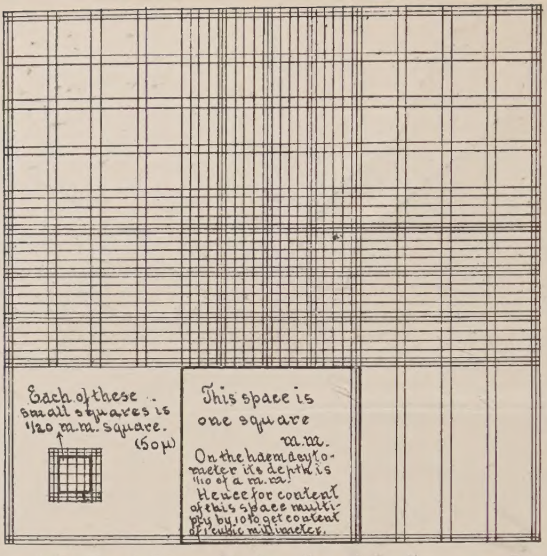

Each of these small squares is 1/20 m.m. square. (50μ)
This space is one square m.m.
On the haemacytometer its depth is 1/10 of a m.m.
Hence for content of this space multiply by 10 to get content of 1 cubic millimeter.

W. B. MEACHAM.

The Ruling of the Turck Disc. Explanation of the Squares.—In the first place, we have the square which encloses the entire ruled surface. This is made up of nine squares, each 1 mm. square. These are the squares to use in connection with leukocyte counts with the white pipette. They may be termed the large squares. The very smallest squares which can be found are those made by the intersection of the triple ruled lines in the center; they are 1/40 mm. or 25 microns square and are never used for any purpose, except possibly in connection with the counting of bacteria in a vaccine. It will be observed that it requires four of these very small squares to make one of the squares usually designated as the small square.

There are 400 small squares in each large square, consequently, as there are nine large squares, the entire ruled surface consists of 3600 small squares. There are 4000 small squares in 1 cubic mm.

The unit in estimating the leukocyte or red cell content of blood is the cubic millimeter. The unit is 1/1000 of a cubic centimeter.

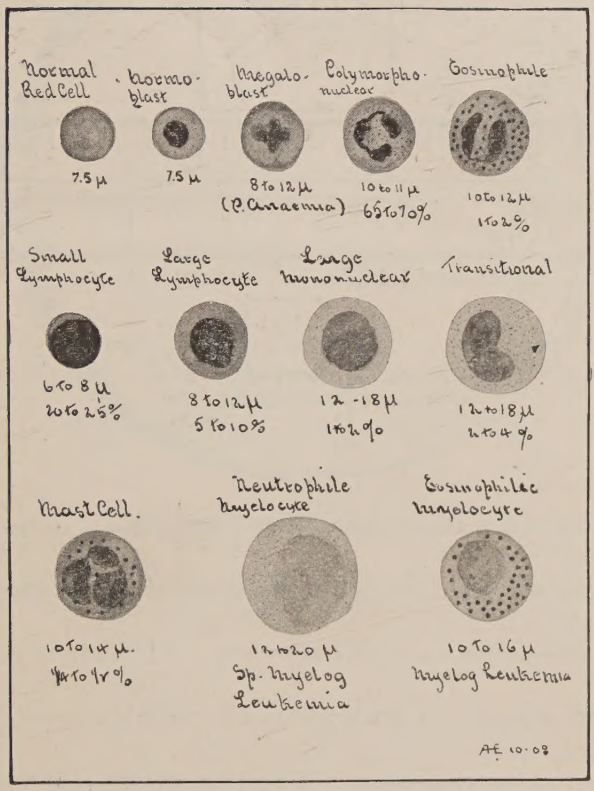
In making a leukocyte count we usually take the white pipette, which has the mark II just above the bulb, and draw up the blood to 0.5 and then with suction we fill the pipette to the II mark with the diluting fluid for which a 1/2% solution of glacial acetic acid in water is most satisfactory. This gives a dilution of 1–20.

Counting with the 2/3 inch objective all of the highly refractile dots representing leukocytes in one of the 1 mm. squares at either of the four corners we note the number and mentally multiply by 20 (the number of times the blood was diluted). As the depth of the diluted blood between the ruled surface of the hæmacytometer slide and the under surface of the cover-glass is only 1/10 of a millimeter, we multiply the figure as above obtained by 10 to get the number of cells in a 1–20 dilution of blood in a space of one cubic millimeter.

Example: Counted 90 leukocytes; $90 \times 20 = 1800 \times 10 = 18{,}000$: equals number of leukocytes in 1 cubic mm. of blood.

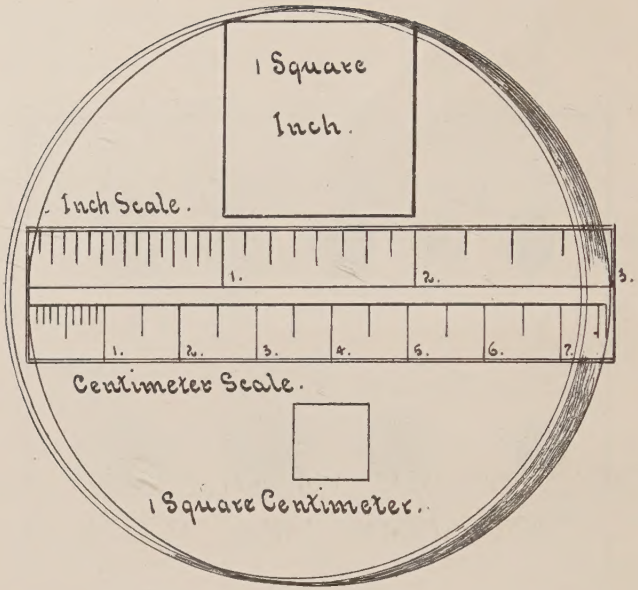
For red counts we use the red count pipette which has the 101 mark just above the bulb. Taking up blood to 0.5 we draw up the diluting fluid to 101. This gives a dilution of 1–200. Counting the red cells in five of the aggregations of 16 small squares (1/20 mm.) thus having counted 80 small squares we have counted 1/50 of the total number of small squares in a cubic mm., there being 4000 small squares in a cubic mm. Consequently the number of red cells in 80 small squares multiplied by 50 and then by the dilution of 200 gives the number of red cells in one cubic mm. of the blood examined.

It is well to make a second preparation and record the average of the two counts.



Principal normal and pathological blood-cells with average size, percentage in a normal differential count and the diseases in which certain pathological cells are more or less pathognomonic.

W. B. MEACHAM.



The diameter of the bottom of this Petri dish is 3 inches or 7.5+ centimeters.

The area of a circle is equal to the square of the radius multiplied by π or 22/7.

1 1/2 in. = radius. 1 1/2 × 1 1/2 = 2.25. 2.25 × 22/7 = 7.07 square inches.

3.75 cm. = radius. 3.75 × 3.75 = 14.06. 14.06 × 22/7 = 44.1 square centimeters.

Number of bacterial colonies in 1 sq. in. averages, approximately, 75. Number in 7.07 sq. in. = 530.

Number of bacterial colonies in 1 sq. cm. averages, approximately, 12. Number in 44.1 sq. cm. = 528.

In a microscopic field, if the diameter were 8 small squares (1/20 mm.), the radius would be 4 small squares and the area of such a round field would be 4 × 4 = 16 × 22/7 = 50+. Such a field would contain 50 small squares.

PRACTICAL BACTERIOLOGY, BLOOD WORK AND ANIMAL PARASITOLOGY

STITT

BY THE SAME AUTHOR

The Diagnostics and Treatment

OF

Tropical Diseases

ILLUSTRATED PREPARING

PRACTICAL

Bacteriology, Blood Work AND Animal Parasitology

INCLUDING

Bacteriological Keys, Zoological Tables and Explanatory Clinical Notes

BY

E. R. STITT, A. B., Ph. G., M. D.

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Third Edition, Revised and Enlarged
With 4 Plates and 106 Other Illustrations Containing 513 Figures

PHILADELPHIA
P. BLAKISTON'S SON & CO.
1012 WALNUT STREET
1914

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THE · MAPLE · PRESS · YORK · PA

PREFACE TO THE THIRD EDITION.

In the preparation of the third edition of this laboratory manual it soon became evident that the new material to be added would increase the size of the book beyond that which would permit its being readily carried in one's pocket. It has, however, been possible to keep the size of the book within the limits considered desirable by the use of a smaller type in a considerable proportion of the paragraphs so that in this way and by increasing the number of lines on each page it has been possible to add extensively to the subject matter and with only an increase of about sixty-five pages.

The advantage attaching to more ready reference obtained by the alternation of different sizes of type would appear to make this plan an improvement over the old.

While the chapters dealing with bacteriology have been added to and made to include more recent advances it will be noted that in the section on animal parasitology the subject matter has been greatly increased.

In the revision of the chapter on protozoa I am greatly indebted to Professor Minchin's recent work on the Protozoa and in those relating to arachnoids and insects to the very practical volume of Colonel Alcock entitled "Entomology for Medical Officers."

The illustrations have been added to and many which did not seem to bring out sufficiently details of anatomy have been replaced by others more satisfactory in that respect.

The three plates of the cestode, trematode and nematode ova were drawn by Mr. L. Avery under the supervision of P. A. Surgeon Garrison, U. S. N. and it is believed that they will be found more satisfactory than similar plates contained in works on animal parasitology.

Several new tables have been added among which special attention is called to the one on urinary findings in various diseases of the genito-urinary system and also to the key to the intestinal bacteria attached to the inside of the board cover.

A chapter on "Disinfectants and Insecticides" giving the practical

application of methods of carrying out these important Public Health questions has been added.

In the chapter on "Immunity" a modification of Emery's technic for the Wassermann test has been incorporated—the use of Noguchi's reagents with Emery's technic. The subject matter of the sections on vaccines and anaphylaxis has been extensively revised.

E. R. S.

PREFACE TO THE SECOND EDITION.

The fact of the necessity for a second edition of this manual of laboratory and clinical diagnosis in a little more than a year would indicate that the original arrangement of material should be adhered to.

Each section of the book had been carefully revised and much new matter added. In particular has that part of the book relating to animal parasitology been rewritten and almost doubled in extent, and a chapter on Poisonous Snakes added. In the chapter on "Practical Methods in Immunity" the most recent advances in the Wassermann test and practical agglutination methods have been incorporated as well as a brief discussion of the question of Anaphylaxis.

The section on "Clinical Bacteriology and Animal Parasitology of the Various Body Fluids and Organs" has been revised to meet the most recent advances in clinical diagnosis. This section not only answers as a cross index to the importance of the various bacteria and animal parasites in practical clinical work, but gives a concise, practical statement as to how to proceed in the examination of various secretions and excretions. This information is difficult to obtain in the larger works on clinical diagnosis by reason of its being taken up under many different headings.

A method is given for the making of differential counts in the same preparation as that for making the leukocyte count which has the advantages of accuracy and the saving of time.

Several new illustrations have been added—the one of poisonous snakes has been taken from Stejneger's report.

The plan of making this little volume a practical one has been continued in the second edition; theoretical considerations have been brought out only when necessary to a proper understanding of some recent or difficult laboratory method.

The very elementary considerations and definitions have not been given because in order to present a compact and at the same time a practical working guide it has been necessary to eliminate that which

seemed least essential. Furthermore, instruction in biological science is now a part of the requirements of candidates for admission to the various medical schools.

At the request of many who have found the book of assistance I have added an outline of those methods in the chemical examination of urine and gastric contents which have seemed to me to be most essential in the making of diagnoses. In the tropics I have found the determinations of total nitrogen and nitrogen eliminated as ammonia to be exceedingly valuable in diagnosis. Methods for such determinations, as elaborated by Assistant Surgeon E. W. Brown, U. S. Navy, of the U. S. Naval Medical School, have proven satisfactory and have been incorporated in this section which is to be found in the Appendix.

Every effort has been made to keep the book within the limits of a pocket manual.

Owing to my absence from the United States I have to thank Dr. Charles S. Butler for correcting the proof. For the revision of the index I am indebted to Mr. John P. Griest.

E. R. S.

PREFACE TO THE FIRST EDITION.

While a member of the Naval Examining Board and examiner in bacteriology and clinical microscopy, I have during the past six years had an opportunity to judge of the qualifications of several hundred graduates of the various medical schools of the country from the standpoint of practical application in the laboratory of that which they had learned as undergraduates.

More particularly I have made it a point to ascertain from the successful candidates, while under instruction at the Naval Medical School, the features of their laboratory courses, which had seemed to them most practical; such methods being subsequently tested in our own class work.

As a result I have endeavored to incorporate in this manual methods which have been submitted to the criticism of postgraduate students from all the leading medical schools of the country, and which have been considered by them adapted to the requirements of practical, speedy, and satisfactory clinical laboratory diagnosis.

For the laboratory worker the most valuable asset is common sense and he must be able to bring to mind the possibilities of the production of various artefacts and results from trivial errors in technic. It has been my object to point out where such mistakes may arise, the reasons for obtaining results differing from those ordinarily obtained and the means employed to eliminate as far as possible such results.

We are too apt to neglect the trivial details of stains, reaction of media, and the like, yet it is only when every detail of technic has been rigidly carried out that we are in a position to judge of the significance of an object observed in a microscopical preparation.

In bacteriology, candidates were frequently able to give the cultural and morphological characteristics of all the important pathogenic organisms, yet when it was required of them to outline the procedure by which they would differentiate members of the typhoid-colon groups when encountered in a plate made from feces, the problem appeared to them impossible. They possessed the information, but did not know how to apply it.

In practical work, organisms can only be separated culturally by the use of Keys and for this reason Keys are given at the beginning of each division of bacteria. These enable one to quickly place the organism isolated in its respective group. Only methods of differentiation which are applicable in a physician's private laboratory are given. Practical methods for making the final identification by agglutination or other immunity tests are described. Technic for immunizing animals to furnish such sera is given in detail.

The giving of the cultural characteristics in a systematic tabulated Key gives space in the notes for presenting the salient points in the pathological and epidemiological aspects of each organism.

I have endeavored to give a scientific yet practical classification of the important pathogenic moulds, a subject about which there exists greater confusion in the minds of students than for any other. In the nomenclature I have followed Gedoelst's "Les Champignons Parasites."

In the chapter on Media Making, it is believed that anyone after reading this section and following the instructions will be able to satisfactorily and without the adjuncts of a large laboratory make any kind of media. The directions as to titrations are given in detail because it is beginning to be recognized that reaction of media in bacteriology is of as great importance as staining is in blood work.

The section on Blood Work is practical and gives a method for making a Romanowsky stain which is quick and reliable. The chapter on Normal and Pathological Blood gives in a few pages the more important points to be borne in mind in considering a possible diagnosis.

While there is no difference between the laboratory requirements of medical work in the tropics and that in temperate climates, unless by reason of such measures of diagnosis being indispensable in the tropics, it has, however, been my endeavor to treat every tropical question, whether in blood work, bacteriology, or animal parasitology, in a more complete way than is usual in manuals of this character. Therefore it is believed that his little book will be of great service to the laboratory worker in the tropics.

It is only from working under Doctor Charles W. Stiles in his course of laboratory instruction in Animal Parasitology in the United States Naval Medical School that I feel justified in presenting a concise outline of the subjects in medical zoology which appear to me to be most important for the physician.

The system of arranging tables, showing the families, genera, etc.,

in which each species belongs will, it is believed, greatly simplify the matter of classification for the medical student. The points given under each parasite are believed to be practical ones. When a parasite has only been reported for man two or three times, very little space is given to it.

Part IV summarizes the various infections which may be found in different organs or excretions of the body and embraces both bacterial and animal parasites. Practical methods for examining material are also given.

The chapter on Immunity, in which the theoretical side is immediately illustrated by the practical application will tend to simplify this bug-bear of the medical student.

The illustrations have been selected with a view to bringing out points which are difficult to state briefly in the text, and furthermore they have been grouped together so that comparison of similar parasites is possible without turning from page to page.

I have in particular to thank Hospital Steward Ebeling of the Navy for his care in bringing out such details.

By reason of the authority of Braun, it has been considered sufficient to give in the tables only the proper zoological name of the parasite as given in the 1908 German edition. The synonyms have been omitted for consideration of space.

The works chiefly consulted in addition to that of Braun have been: Albutt's System of Medicine; Osler's System of Medicine; Muir and Ritchie's Bacteriology; Mense's Tropenkrankheiten; Blanchard's Les Moustiques; Guiart and Grimbert's Diagnostic; Ehrlich's Studies in Immunity; Stephens and Christopher's Practical Study of Malaria; Daniel's Laboratory Studies in Tropical Medicine; Manson's Tropical Diseases; Gedoelst's Les Champignons Parasites; Neveu-Lemaire Parasitologie Humaine; Chester's Determinative Bacteriology; Lehmann and Neumann's Bacteriology.

E. R. S.

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BACTERIOLOGY, BLOOD-WORK AND ANIMAL PARASITOLOGY.

CHAPTER I.

APPARATUS.

The Microscope.

The most important piece of apparatus for the laboratory worker is the microscope. Very satisfactory microscopes can be purchased in this country. Instruments of standard German make are in use in many laboratories and appear to give general satisfaction. It is impossible to do good microscopical work unless the microscope gives and continues to give good definition and the working parts remain firm. Folding microscope stands are now made which are perfectly satisfactory, such instruments, however, have only the advantage of occupying, less space in a case so that unless the question of compactness is involved, as in an outfit for the military services or for a microscopist who travels about a great deal, the ordinary rigid horseshoe base is to be preferred.

A mechanical stage is almost a necessity in connection with blood-work and its use is advantageous in bacterial preparations. For the study of tissue sections the moving of the slide with the fingers is preferable. Therefore, the mechanical stage should be capable of ready attachment or removal. For the examination of colonies growing in Petri dishes we also use the stage unencumbered with the mechanical stage. A triple or quadruple nose-piece, according to the number of objectives used, is also indispensable.

Objectives.—To meet the demands of clinical microscopy there should be three objectives, preferably a 16-mm. (2/3-in.), a 4-mm. (1/6-in.) and a 2-mm. (1/12-in.) homogeneous oil immersion. The Zeiss AA is a 17-mm. objective, and the Leitz No. 3, an 18-mm. one. The Zeiss D is about 4.2-mm. and the Leitz No. 6, a 4.4-mm. A dust-proof quadruple nose-piece with four objectives will be found a great convenience (in addition to the 2/3-in. and 1/12-in. objectives, a 1/4-in.

for urine and blood counting, with a 1/8-in. for examining hanging-drop preparations and for quick examination of blood smears). An apochromatic objective costs about three times as much as an achromatic one and, except in photographic work, has little if any advantage.

As regards oculars (eye-pieces) a No. 2 and a No. 4 will best meet the requirements. For high magnification a No. 8 may be of service. The Zeiss oculars are numbered according to the amount they increase the magnification given by the objective; thus a No. 2 increases the magnification, given by the objective alone, twice; a No. 8, eight times. Some oculars are classified according to the equivalent focal distance, and are referred to as 1/2-in., 1-in., and 2-in. oculars.

The oculars in common use are known as negative oculars, by which is meant an ocular in which the lower lens (collective) assists in forming the real inverted image which is focused at the level of the diaphragm within the ocular. When using a disc micrometer, it is supported by this diaphragm, and the outlines of the image are cut by the rulings on the glass disc, and so we are enabled to measure the size of the object examined. The measurement of various bacteria, blood-cells, and parasites is exceedingly simple and assists greatly in the study of bacteria, and is indispensable in work in animal parasitology. (For details of micrometry see section on blood-work.) When an ocular is termed positive, it refers to an ocular which acts as a simple microscope in magnifying the image, the image being formed entirely by the objective and being located below the ocular.

Objectives are usually designated by their equivalent focal distance. It is important to remember that the equivalent focal distance does not represent the working distance of an objective, by which is meant the distance from the upper surface of the cover-glass to the lower surface of the objective. Thus a 1/4-in. objective may have to be approached to the object so that the distance intervening may be only 1/6 in. or even less. This explains the frequent inability to focus an object when a high-power dry objective (1/6-in. or 1/8-in.) is used with a rather thick cover-glass—the objective possibly having a short working distance, so that the thickness of the cover-glass does not allow of any free working distance.

Instrument makers generally specify the thickness of cover-glass to be used with a certain tube length, but as a practical matter it will be found convenient to use No. 1 (very thin) cover-glasses. The principal objection to these is that they are more fragile than the No. 2, but with a little practice in cleaning cover-glasses this is negligible. Immersion lenses are less affected than dry lenses by the question of a certain thickness of cover-glasses for a certain tube length.

One of the most fruitful causes of the crushing of microscopical objects and the overlying cover-glass or, what is far more important, the breaking of the cover-glass of a hanging-drop preparation and consequent risk of infection is the attempt to focus with the fine adjustment. It should be an invariable rule for the worker to bring his object-

ive practically into contact with the upper surface of the cover-glass, then using the coarse adjustment (rack and pinion) to slowly elevate the objective, looking through the eye-piece at the same time. In other words, obtain focus with the coarse adjustment and maintain it with the fine adjustment (micrometer screw). The fine adjustment should only be used after the focus is obtained.

In using the oil-immersion objective always dip the lens in the oil and practically touch the cover-glass—the eye being at a level with the stage—before beginning to focus. With the coarse adjustment one can feel the contact with the cover-glass, which is impossible with the fine adjustment. It saves time and disappointment to make a preliminary examination of a preparation requiring the high dry or immersion lens with a low power (2/3-in.) before employing the higher power; in this way we locate or center a suitable field for study.

It will be observed that objectives frequently have their numerical aperture marked on them. This is expressed by the letters N.A. From a practical standpoint this gives the relative proportion of the rays which proceeding from an object can enter the lens of the objective and form the image. Of course, the greater the number of rays, the greater the N.A., the better the definition, and consequently the better the objective. Immersion oil, having the same index of refraction (1.52) as glass, would not deflect rays coming from the object and so prevent their entering the objective, as would be the case if we used a dry objective with an intervening air space. In this case a portion of the rays would be turned aside by the difference in the refractive index of air. As a rule, the higher the numerical aperture, the better the objective and the less the working distance. In blood counting, the cover-glass being comparatively thick, it may happen that with a 1/6-in. of high numerical aperture there may not be sufficient working distance to bring the blood-cells into focus, which could be done with an objective of lower numerical aperture. Consequently, we must always consider the matter of working distance as well as that of numerical aperture. The skill of the optician, however, can obviate this defect in an objective of high numerical aperture so that it may combine the qualities of perfect definition with sufficient working distance.

Practical Points in the Use of the Microscope.—An important matter in the use of the microscope is to get all the details possible with a low power before using a higher power. This, of course, does not apply to a bacterial preparation where it is necessary to use a 1/12-in. or a high-power dry lens. It is well, however, in a bacterial or blood preparation to first examine the smear with the 2/3-in. objective in order to determine suitable areas for examination with the oil-immersion objective. With tissue sections it is not only advisable to begin the study with the lowest power, but even an examination with the unaided eye or with a magnifying glass, before using the microscope, will give a surprising amount of information.

After using the oil-immersion objective the lens should be wiped clean of oil with a strip of Japanese lens paper or with a silk handkerchief. If the oil should dry on the surface of the lens it may be removed with a drop of xylol on a piece of lens paper. Immediately afterward the lens should be dried. Dried oil on a lens often causes the lens to be considered defective. Accidental contact of the dry objectives with oil is not uncommon and should always be thought of when satisfactory optical effects are not obtainable.

It is advisable to cultivate the use of both eyes in doing microscopical work. When using one eye the other should be kept open with accommodation relaxed. It is this squinting of the unemployed eye which so often fatigues. A strip of cardboard 4 or 5 inches long, with an opening to fit over the tube of the microscope, leaving the other end to block the vision of the unused eye, will prevent the strain. This apparatus can be purchased in vulcanite.

A warm stage for the study of living protozoa may be extemporized by taking a piece of copper about the size of the stage and with a strip projecting out anteriorly for 5 or 6 inches. The under surface of the plate is covered with flannel and a hole about 1 inch in diameter cut out of the center. The proper amount of heat is applied by a flame impinging on the tongue-like projection of the copper plate.

Direct sunlight or excessively bright light is to be avoided. If such conditions must exist a white shade or muslin curtain drawn across the window is a necessity. Light from the north and from a white cloud is the most desirable. South of the equator a southern light. In the tropics a piece of plate glass fitted into the lower part of a wire screen frame gives good lighting, keeps out dust, and does not interfere greatly with the circulation of the air.

The technic in connection with proper illumination is probably more important than any other point; unless the light is utilized to the best advantage, the best results cannot be obtained. In examining fresh blood preparations or hanging drops the concave mirror should be used and the light almost shut off by the iris diaphragm so as to give a contour picture. In examining a stained blood or bacterial preparation, the Abbe condenser should be properly focused so as to best illuminate the stained film. In many instruments set-screws are provided which check the elevation of the Abbe condenser when the proper focus is reached. Inasmuch as the light from the condenser should come to a focus exactly level with the object studied, it is evident that a fixed position for the condenser would not answer when slides of different thickness were used. Always use the plane mirror when examining stained bacterial or blood films, as a color image is desired. Ordinarily in examining tissue sections, the Abbe condenser should either be put out of focus by racking down or by the use of the concave mirror and the narrowing of the aperture of the iris diaphragm. Swing-out condensers are now made which are very convenient. The proper employment of illumination only comes with experience, and one should continue to manipulate his mirrors, diaphragm, and condenser until the best result is obtained. Then study the specimen.

For microscopical work in a laboratory not properly supplied with windows or for night work the frosted incandescent bulb is very satisfactory.

Dark Ground Illumination.—Very valuable information, especially as regards the detection of treponemata in material from hard chancres

or mucous patches, may be obtained by the use of dark ground illumination. There are many different types of apparatus for this purpose.

The bacteria or spirochætes are intensely illuminated and show as brilliant silvery objects in contrast to the dark background.

When the morphological details of a brightly illuminated object in the dark field can be distinctly observed it is proper to use the term dark ground illumination. When only particles, usually surrounded by bright and dark rings, and not showing any structure, are observed in the dark field the proper designation is ultramicroscopic. An apparatus using only the short waves of the ultra-violet spectrum enables one to observe particles no larger than 1/10 of a micron. For this apparatus it is necessary to employ photographic plates. In using the 1/12-inch objective with dark ground illumination a funnel-like base is supplied on which we screw the nickle plated front mount of the objective. Before using the dark-field apparatus it must be centered with a low power. This is carried out by getting concentric rings parallel with the circle of the microscopic field. Immersion contact between the front surface of the Abbe condenser and the under surface of the slide carrying the preparation must be made before focussing the 1/12th objective. As a source of illumination we may use a small arc-lamp or a Nernst lamp or an incandescent gas lamp. In using an arc-lamp one must have a suitable rheostat according to the electrical current employed. Information as to voltage and nature of current must be given the one supplying the apparatus.

In making preparations the slides and cover-slips should be scrupulously clean and the material thinly spread out and free of bubbles.

Apparatus for Sterilization.

For the purpose of sterilizing glassware, media, and old cultures there are three methods ordinarily employed. The hot-air sterilizer, in which a temperature of about 150° C. is maintained for one hour, is ordinarily used for the sterilization of Petri dishes, test-tubes, pipettes, etc. If the temperature is allowed to go too high, there is danger of charring the cotton plugs and also of causing the development of an empyreumatic oil which makes the plugs unsightly and causes them to stick to the glass. Again we must be careful not to open the door until the temperature has fallen to 60° C., otherwise there is danger of cracking the glassware. Where gas is not obtainable, the hot-air sterilizer is not a very satisfactory apparatus.

The Arnold sterilizer is to be found everywhere and can be used on blue-flame kerosene-oil stoves as readily as with gas burners. The most convenient form, but more expensive, is the Boston Board of Health pattern. The ordinary pattern, with a telescoping outer portion, answers all purposes, however. In the Arnold, sterilization is

effected by streaming steam at 100° C. It is usual to maintain this temperature for fifteen to twenty-five minutes each day for three successive days. The success of this procedure—fractional sterilization—is due to the fact that many spores which were not killed at the first steaming have developed into vegetative forms within twenty-four

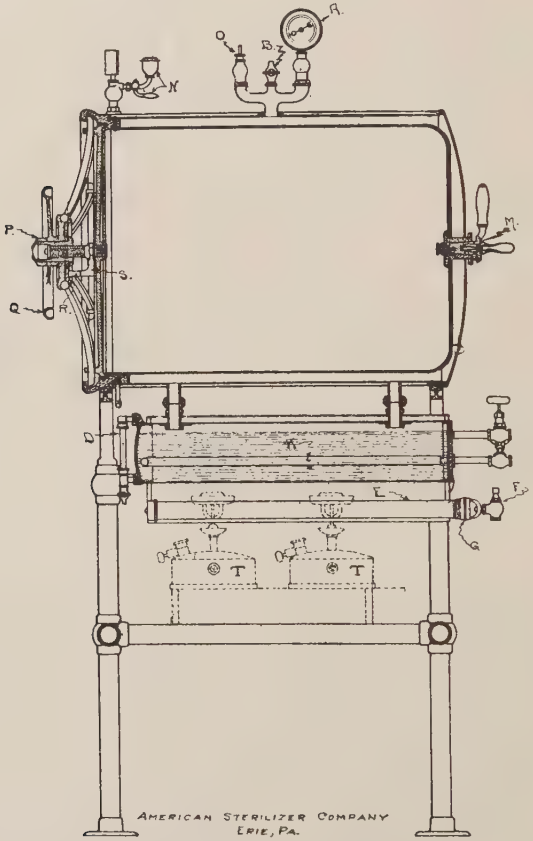

FIG. 1.—Dressing sterilizer showing cylinder containing water (K) heated either by gas or Primus kerosene lamps.

hours, and when the steam is then applied such forms are destroyed. Experience has shown that all the spores have developed by the time of the third steaming, so that with this final application of heat we secure perfect sterilization.

It is customary to use the Arnold for sterilizing gelatin and milk

media, even when the autoclave is at hand, the idea being that the greater heat of the autoclave may interfere with the quality of such media. The most convenient autoclave is the horizontal type, such as is to be found everywhere for the sterilization of surgical dressings.

The source of heat may be either gas, the Primus kerosene-oil lamp or steam from an adjacent boiler. More recently a method of employing kerosene, gasolene, or alcohol with a gravity system has been perfected. During the past 6 years, in the laboratory of the U. S. Naval Medical School, we have been using a dressing sterilizer, made by the American Sterilizer Co., with which it has been possible to most satisfactorily carry out all kinds of sterilization, thus doing away with the use of the Arnold and the hot-air sterilizer. It is impossible to sterilize ordinary fermentation tubes in the autoclave on account of the boiling up of the media and wetting of the plugs. This is still done with the Arnold. By use of the Durham tubes—which are to be preferred, except for gas analysis—sugar media can be thus sterilized.

Should a small bubble remain in the top of the small inverted inner tube after removal from the autoclave, one may make a mark with a grease pencil at the line of the bubble; or, if preferred, the basket of Durham tubes can be heated to boiling for ten minutes in a pan of water or in the Arnold when, after cooling, the bubble will be found to have disappeared.

Glassware will come out from such an autoclave with wrappers as dry and plugs of the test-tubes as stopper-like as could be effected in a hot-air sterilizer.

The objection which exists in the use of some autoclaves, as regards condensation on dressings or apparatus, does not exist in this type. The mechanism, by which the inner and outer chambers are connected and disconnected, and that for vacuum production, rests in the simple turning of a lever from mark to mark. We have been able with a gas burner to obtain a pressure of 15 pounds in less than ten minutes. In sterilizing test-tubes we place them in small rectangular wire baskets, $6\times5\times4$ in. These baskets are to be preferred to round ones, as they pack more satisfactorily in the refrigerator used for storing media. In sterilizing flasks, test-tubes, Petri dishes, throat swabs, pipettes, etc., it has been our custom, after exposing to 20 pounds' pressure for twenty minutes, to produce a vacuum for two or three minutes; then with the steam in the outer jacket for a few minutes to thoroughly dry the articles in the disinfecting chamber. The valve to the inner chamber is then opened to break the vacuum; the door is now opened, and the articles removed in as dry a state as if they had been in the hot-air sterilizer. Articles, however, can be thoroughly dried without the use of a vacuum, simply allowing the steam to remain in the outer jacket with the steam cut off from the inner chamber.

PRESSURE AND TEMPERATURE TABLE.

5 pounds' pressure,	107.7° C.,	226° F.
10 pounds' pressure,	115.5° C.,	240° F.
15 pounds' pressure,	121.6° C.,	250° F.
20 pounds' pressure,	126.6° C.,	260° F.
25 pounds' pressure,	130.5° C.,	267° F.
30 pounds' pressure,	134.4° C.,	274° F.

All such articles as Petri dishes, pipettes, swabs, etc., are wrapped in cheap quality filter-paper, making a fold and turning in the ends as is done in a druggist's package. Old newspapers answer well for this purpose. The sterile swab can be used for many purposes in the laboratory. They are most easily made by taking a piece of copper wire about 8 inches long, flattening one end with a stroke of a hammer, then twisting a small pledget of plain absorbent cotton around the flattened end. After wrapping, the swabs are sterilized in bunches. We not only use them for getting throat cultures, but in addition for culturing fæces, pus, or other such material. The pus is obtained with a swab, which material is then distributed in a tube of sterile bouillon or water. With the same swab the surface of an agar plate is successively stroked. This method is almost as satisfactory as the German one of using bent glass rods for this purpose. Everyone has encountered the difficulties attendant upon the bending of platinum wires and also the possibility of destroying your organisms by an insufficiently cooled wire.

Cleaning Glassware.

It is a routine in our laboratory for everything to go through the sterilizer at 125° C. before anything else is done. This is a safe rule when dealing with dangerous pathogenic organisms (especially tetanus and anthrax).

As soon as taken out of the sterilizer the contents are emptied, and the tube or dishes placed in a 1% solution of washing soda and boiled. This thoroughly cleans them. As the washing soda slightly raises the boiling-point and also makes the spores more penetrable, it would appear that under ordinary circumstances, it would be sufficient to place all contaminated articles in a dishpan with the soda solution, and boil for at least one hour, not using a preliminary sterilization in the autoclave. The tubes are now cleaned with a test-tube brush, thoroughly rinsed with tap water and placed in a 1% solution of hydrochloric acid for a few minutes; then rinsed thoroughly in water and placed in test-tube baskets, mouth downward, and allowed to drain over night. Some laboratory workers boil their test-tubes and other glassware in water containing soap or soap powder and, after a thorough rinsing in tap water, drain. Hydrochloric acid should not be used after the soap as it will cause the formation of an unsightly coating difficult to remove. When thoroughly dry they may be plugged and sterilized. To plug a test-tube, pick out a little pledget of plain absorbent cotton about 2 inches in diameter from a roll. Place it over the center of the tube and with a glass rod push the cotton down the tube about an inch. The cleaning fluid commonly used in laboratories consists of one part each of potassium bichromate and commercial sulphuric acid with ten parts of water. This is an excellent mixture for cleaning old slides, etc., especially when grease or balsam is to be gotten rid of. It is very corrosive, however. An efficient and less corrosive method for cleansing slides and cover-glasses is to leave them over night in an acetic acid alcohol mixture (two parts of glacial acetic acid to one hundred parts of alcohol). After drying and polishing out of this mixture,

it is well to pass the slides and cover-glasses through the flame of a Bunsen burner or alcohol lamp to remove every vestige of grease. Ordinarily, rubbing between the thumb and forefinger with soap and water, then drying with an old piece of linen, and finally flaming will yield a perfect surface for making a bacterial preparation.

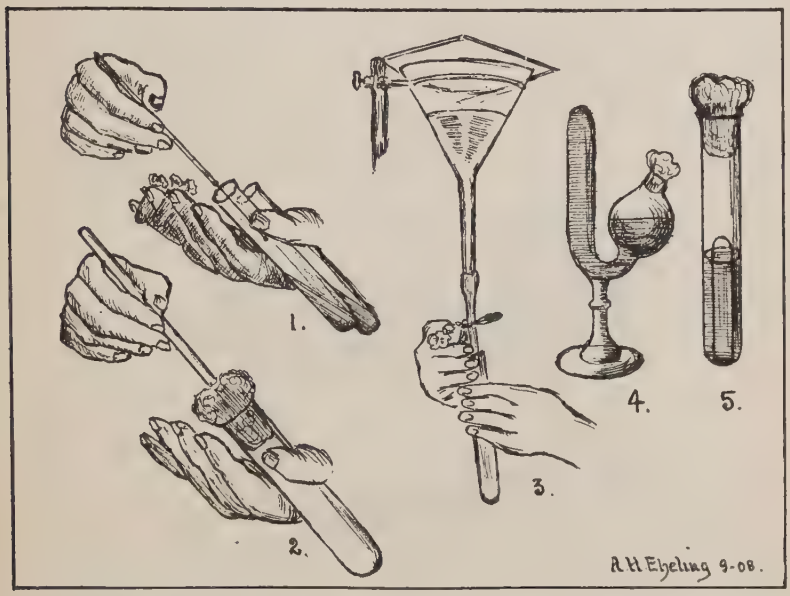

FIG. 2.—1, Inoculation of tubes; 2, plugging of tubes; 3, filling tubes; 4, Smith's fermentation tube; 5, Durham's fermentation tube.

CONCAVE SLIDES, FERMENTATION TUBES.

The concave slide is ordinarily used for making hanging-drop preparations for the examination of bacteria as to motility, capsules, size and arrangement. To prepare a hanging-drop preparation for the study of motility it is best to place a loopful of the young bouillon

FIG. 3.—Hanging drop, over hollow ground slide. (*Williams.*)

culture or a loopful of salt solution into which is then emulsified a small amount of growth from an agar slant, in the center of the cover-glass; now having applied with a brush a ring of vaseline around the

concave depression in the slide we apply the slide as a cover to the cover-glass which latter adheres to the ring of vaseline. The completed hanging-drop preparation can now be turned over and placed on the stage of the microscope.

A substitute which is equally good may be made by spreading a ring or square of vaseline—smaller than the cover-glass to be used—in the middle of a plain slide. Then putting a loopful of salt solution in the center of the space, and inoculating with the culture to be studied, we finally cover it with a cover-glass, gently pressing the margins down on the vaseline. This gives a preparation for the study of motility or agglutination which does not dry out for hours, and is easier to focus upon than the concave slide hanging-drop preparation

In examining a hanging drop first use a low-power objective and, having brought

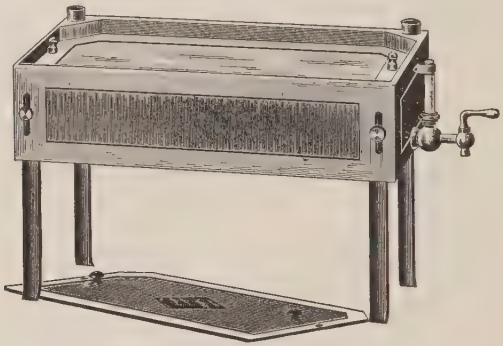

Fig. 4.—Blood serum coagulating apparatus.

into focus the margin of the drop as a center line, change to a 1/6- or 1/8-in. objective. By this procedure a thin layer of fluid is brought under the high dry objective instead of the deeper layer in the center of the drop. It is not advisable to use an immersion objective with a hanging-drop preparation.

The light should be cut down to a minimum with the iris diaphragm and the concave mirror used. When we have finished examining the preparation the cover-glass should be pushed over with the forceps so that a corner projects and we then seize this with the forceps, lift up the cover-glass and drop it into the disinfecting solution along with the slide.

The fermentation tube with a bulb and closed arm is expensive, difficult to clean, and is easily broken. It is, however, convenient in the determination of the gas formula of an organism. It's use is described under water analysis. As a substitute in the study of gas production and in water bacteriology, the Durham tube is to be recommended.

Into a test-tube, about 1×7 in., we introduce the special sugar media, then drop down a small test-tube (1/2×3 in.) with its open end downward. Insert the

plug of the large tube and sterilize. During sterilization the fluid enters the mouth of the smaller tube and fills it, and when the medium is subsequently inoculated, if gas forms, it appears in the upper part of the closed end of the smaller tube.

For inspissating blood-serum slants a regular inspissator is desirable. This is nothing more than a double-walled vessel, the space between the walls being filled with water.

As a substitute one may take the common rice cooker (double boiler). Fill the outer part with water; and in the inner compartment pack the serum tubes properly slanted on a piece of wood or a wedge-shaped layer of cotton. Place a weight on the cover of the inner compartment to sink it into the surrounding water, and allow to boil for one or two hours. This same apparatus may be used for their sterilization on two subsequent days, but it is better to sterilize in the autoclave or Arnold. As regards a working desk, it will be found convenient to have an arrangement similar to the ordinary flat-top desk, with a tier of drawers on each side. A block of wood with holes bored in it to contain dropping-bottles may be placed in the upper lefthand drawer. In this way the stains are as accessible as if they encumbered the desk. It is advisable to paint the inside of this drawer black so that the light may not cause the staining reagents to deteriorate.

FIG. 5.—Rice cooker.

A very popular method of preparing the surfaces of laboratory desks, sinks, and tables is the application of the so-called "acid-proofing." This gives an ebony-like finish which is not affected by strong acids.

In using it the surface of the wood must be new (free of any varnish, oil, or paint; if previously so coated the surface must be planed).

Solution 1.

Potassium chlorate,	125.0 gms.
Cupric sulphate,	125.0 gms.
Water,	1000.0 c.c.

Apply two coats of this solution at least 12 hours between applications. When thoroughly dry apply two coats of solution No. 2.

Solution 2.

Aniline oil,	120.0 c.c.
Hydrochloric acid,	180.0 c.c.
Water,	1000.0 c.c.

When the treated surface is thoroughly dry apply one coat of raw linseed oil with a cloth. After this is dry wash with very hot soapsuds.

An aspirating bottle on a shelf elevated two feet, with rubber tubing and glass tip leading to a small aquarium jar or other desk receptacle, makes a good substitute for a small sink and faucet. A Hoffman screw clamp on the rubber tube controls the flow of water.

Ordinary glass salt cellars will be found very useful, where the watch-glass is employed. They may also be wrapped, sterilized, and used to contain fluids for inoculating, etc.

A glass-topped fruit jar or a specimen jar containing a disinfecting solution for contaminated slides, etc., should be on every working desk. A good solution is that of Harrington (corrosive sublimate, 0.8; commercial HCl, 60.0 c c.; alcohol, 400.0 c.c.; water, to 1000.0 c.c.).

A very simple method of making a disinfectant similar to lysol is to put one part of cresol or crude carbolic acid and one part of soft soap in a wide-mouthed bottle over night. The resulting compound makes a perfect solution with water and a 5% solution of this will be found at least equal to a 5% phenol solution. In addition to using as a desk jar disinfectant it is excellent for disinfecting fæces, sputum, etc.

For use in making loops and needles, platinum wire of 26 gauge will be found most suitable. The handle made of glass rod is preferable to the metal ones. One end is fused in the flame and, holding the 3- to 4-in. piece of platinum wire, with forceps, in the same flame, insert the glowing metal into the molten glass.

For making smears from fæces, sputum, and the like, wooden tooth-picks are very convenient; the kind with the spatulate end is preferable.

When gas is obtainable, the maintaining of a constant temperature for the body temperature incubator (38° C.) and the paraffin oven (60° C.) is best secured by the use of some of the various types of thermo-regulators. The Reichert type is the one in general use, although there are many features about the Dunham and Roux regulators which are advantageous.

If the pressure of the gas-supply varies from time to time, it is essential to regulate this by the use of a gas-pressure regulator (Murrill's is a cheap and satisfactory one).

Incubators, controlled electrically, can be obtained of certain foreign makers, and are quoted in catalogues of American dealers. It is probable that the Koch petroleum lamp incubator is the most satisafctory one where gas is not obtainable. They should be of all metal construction, and not with a wood casing, on account of the danger from fire. They cost from twenty-five to fifty dollars.

An incubator may be extemporized by putting the bulb of an incandescent electric lamp in a vessel of water. The proper temperature may be obtained by increasing the amount of water or by covering the opening more or less completely with a towel. The test-tubes to be incubated can be put into a fruit jar or tin can, which receptacle is placed in the vessel heated by the lamp.

Emery suggests the use of a Thermos bottle as an incubator.

The vacuum bottle should be first warmed by pouring in warm water. Afterward the bottle should be three-fourths filled with water at 100° F.

Schrup suspends his cultures and thermometer in the water by threads attached to pins in the cork of the vacuum bottle. The plug should be paraffined or covered with a rubber cap. As regards the matter of a low-temperature incubator (for gelatin work), this is best met by using a small refrigerator. The ice in the upper part maintains an even cold, and by connecting up an electric lamp in the lower part of the refrigerator we can easily maintain a temperature which only varies one or two degrees during the twenty-four hours.

With a 16-candle-power lamp a temperature of about 25° C. is maintained (this is too high, being about the melting-point of gelatin); with an 8-candle-power, one

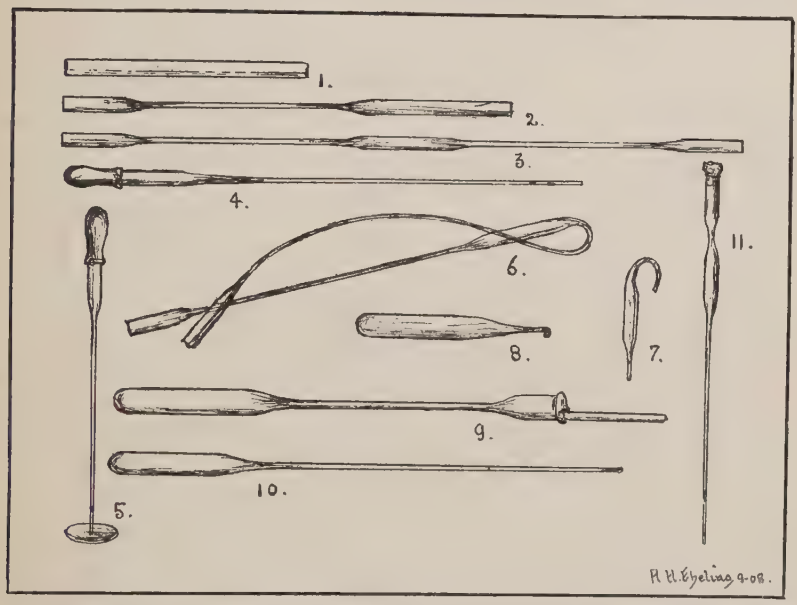

FIG. 6.—1, 2, 3, Drawing out glass tubing; 4, 5, Wright's rubber bulb capillary pipettes showing grease pencil mark for making dilutions; 6, 7, Wright's U-tubes; 8, 9, 10, methods of drawing out test-tubes for vaccines in opsonic work; 11, bacteriological pipette.

about 21° to 23° C.; and with a 4-candle-power, from 18° to 20° C.; the box being about 20×30×36 inches.

When much serum reaction work is done, an electrically run centrifuge is a great convenience.

A filter pump attached to the water faucet, preferably by screw threads, is almost indispensable for filtering cultures, etc., and for cleaning small pipettes, especially the hæmocytometer pipettes. Such a filter or vacuum pump with a vacuum gauge is more easily controlled.

The filter pump is indispensable when using the various types of porcelain or Berkefeld filters. The Punkal or Muencke types of filter are the most convenient in

filtering toxins or in the sterilization of certain media when heating would be unadvisable.

With the possible exception of the platinum loop, there is no piece of apparatus so applicable to many uses as the capillary pipette made from a piece of glass tubing.

These may be made in a great variety of shapes. The one with a hooked end, the Wright tube, is the best apparatus for securing blood

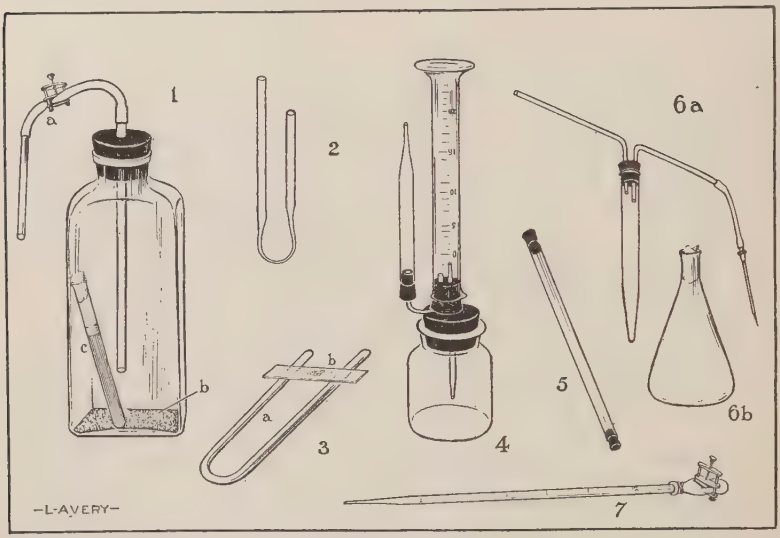

Fig. 7.—1, Apparatus combining various methods for culture of anaerobes; (*a*) Hofmann clamp for connecting with vacuum pump; (*b*) pyrogallic at bottom of bottle for Buchner's O absorption method; (*c*) deep glucose agar stab covered with sterile liquid petrolatum (see anaerobes). 2, One-fourth inch capillary loop U tube for making two nitric acid albumin tests (see chemical examination of urine). 3, Piece of tubing bent to hold slide for steaming smears in flame. 4, Schmidt's fermentation apparatus, as modified by using graduated cylinder (see under fæces). 5, One-fourth inch glass tubing, 4 1/2 inches long with corks at each end. For centrifuging fæces for ova. 6*a*, Apparatus connected with sterile centrifuge tube for taking blood from vein of man or a guinea-pig or rabbit's heart. 6*b*, Erlenmeyer flask which can be used instead of centrifuge tube. See under sections Immunity and Blood. 7, A graduated pipette with Hofmann clamp applied to rubber bulb for precise delivery of measured quantities of liquids.

for serum tests. The crook hangs on the centrifuge guard and by filing and breaking the thicker part of the tube the serum is accessible to a capillary rubber bulb pipette or to the tip of a hæmocytometer pipette. In this way dilutions of serum are easily made. The capil-

lary pipette is made by taking a piece of 1/4-in. soft German glass tubing, about 6 inches long, and heating in the middle in a Bunsen flame, revolving the tubing while heating it. When it becomes soft in the center, remove from the flame and with a steady even pull separate the two ends. The capillary portion should be from 18 to 20 inches in length. When cool, file and break off this capillary portion in the middle. We then have two capillary pipettes. By using a rubber bulb, such as comes on medicine droppers, we have a means of sucking up and forcing out fluids by pressure with the thumb and forefinger of the right hand. The bulb should be pushed on about 1/2 to 3/4 in.; this gives a firmer surface to control the pressure on the bulb.

A bacteriological pipette is made by drawing out a 9-inch piece of tubing about 3 inches at either end, then heating in the middle we draw out and have two pipettes similar to the one shown in the drawing. A piece of cotton is loosely pushed in just above the narrow portion. These may be wrapped in paper and sterilized for future use. They may be made perfectly sterile at the time of drawing out.

Where gas is not at hand, the Barthel alcohol lamp gives a flame similar to that of the Bunsen lamp and is equally satisfactory for heating glass tubing. By making a collar with a lateral opening to fit the burner of a Primus lamp a powerful side-flame is obtained which is almost as suitable for glass blowing as the Bunsen blast usually employed.

CHAPTER II.

CULTURE MEDIA.

While there are certain advantages in sterilizing the glass test-tubes prior to filling them with media, yet this may be dispensed with—the sterilization after the media has been tubed being sufficient. If a dressing sterilizer is at hand, this is preferable for sterilizing such media as bouillon, potato, and agar (10 to 15 pounds' pressure for fifteen minutes). Milk should be sterilized with the Arnold, subjecting the media to three steamings for twenty minutes on three successive days. Gelatin may be sterilized in either way, but preferably in the autoclave at 7 pounds' pressure for fifteen minutes. As soon as taken out of the sterilizer it should be cooled as quickly as possible in cold water. This procedure tends to prevent the lowering of the melting-point of the finished gelatin and also preserves its spissitude.

Blood-serum is preferably solidified as slants in a blood-serum inspissator. This requires one to two hours. The subsequent sterilization in the autoclave or Arnold should not be done immediately after making the solidified slants, but on the subsequent day. If done on the same day, many of the slants are ruined by being disrupted by bubbles. The preparation of blood-serum slants or slants of egg media can be conveniently carried out in a rice cooker (double boiler). Place the tubes in the inner compartment of the cooker, obtaining the slant desired by manipulating an empty test-tube, or with a towel or cotton batting on the bottom. Then cover the tubes with another towel. The outer compartment should contain water alone (no 25% salt solution). The inner compartment should be weighted down so that it is surrounded by water—the light tubes not being sufficient to sink it. Allowing the water in the outer compartment to boil one or two hours will inspissate or solidify the slants satisfactorily. The sterilization on subsequent days may be carried out in the same apparatus, although it is more efficient if done in an Arnold or an autoclave. (This sterilization in the rice cooker makes the media too dry.)

In making media a rice cooker is almost essential; at any rate, it is so if ease, expedition, and unfailing success in preparation are to be achieved. As it is necessary to make the contents of the inner compartment boil, the temperature of the water in the outer compartment must be raised. This is done by using a 25% solution of common salt or a 20% solution of calcium chloride in the outer compartment instead of plain water. Should $CaCl_2$ be carried over to media in inner compartment (as by thermometer) coagulation of albumin and clearing of medium will be prevented.

A 15% solution of salt raises the boiling-point 2 1/2° C.; a 20%, 3 1/2° C., and a 25%, 4 1/2° C. The raising of the boiling-point by calcium chloride is about the same for similar strength solutions.

Although the Bacteriological Committee of the A P. H. Association recommends special steps to be taken in the preparation of gelatin and agar, yet for clinical purposes it will be found satisfactory to keep on hand a stock of bouillon, and when it is desired to make agar or gelatin to simply prepare such media from the stock bouillon in the way to be subsequently given.

Nutrient Bouillon.

This may be made either from fresh meat or from meat extract. Media from fresh meat are usually lighter in color and possibly clearer. In the Philippines, however, certain measures employed for the preservation of the meat made it very difficult to prepare clear bouillon from it, so that meat extract was used entirely. There is very little difference, if any, in the nutritive power of media made in either way. The chief objections to fresh meat as a base are: 1. It takes more time and trouble. 2. The reaction, due to sarcolactic acid and acid salts, is quite acid, so that it is necessary to titrate and neutralize the excess of acidity. 3. The reaction of the finished media tends to change unless the boiling at the time of making was very prolonged. 4. It is not infrequent to have a heavy precipitate of phosphates thrown down at the time of sterilization, thus making it necessary to repeat the process of filtration and sterilization.

If fresh meat is used, take about 500 grams (one pound), remove fat and cut it up with a sausage mill or purchase the meat already cut up as for a Hamburg steak. It makes little difference whether the amount be 100 grams more or less. Place the chopped-up meat in a receptacle and pour 1000 c.c. of water over it. Keep in the ice chest over night and the next morning skim off with a piece of absorbent cotton the scum of fat; then squeeze out the infusion with a strong muslin cloth, making the amount up to 1000 c.c. This meat infusion contains all the albuminous material necessary for the clarification of the bouillon. It is convenient to designate this meat base as Meat Infusion to distinguish from the base containing meat extract.

Having obtained 1000 c.c. of this 50% meat infusion, we dissolve in it 1% of Witte's peptone and 1/2% of sodium chloride. While there is a sufficiency of the various salts necessary for bacterial development in the meat juices, yet there is not enough to give the best results when bouillon cultures of various organisms are used for agglutination tests; and furthermore, when bouillon is used for blood cultures, disintegration of the red cells, with clouding of the clear medium, may occur if there be not sufficient salt present to prevent this.

The salt and the peptone are best put in a mortar, and adding about one ounce of the meat infusion we make a pasty mass; then we gradually add the remaining infusion until solution is complete. It is sometimes recommended to use a temper-

ature of 50° C. to facilitate the solution of the peptone. This is not necessary, and if the temperature is not watched closely it might go up to 65° C. or higher and we should lose the clearing albuminous material from its coagulation. Of this rather cloudy solution take up 10 c.c. with a pipette and let it run out into a porcelain dish. Add 40 c.c. of distilled or rain water and about six drops of a 0.5% phenolphthalein solution. (Phenolphthalein, 0.5; dilute alcohol, 100 c.c.) Bring the contents of the porcelain dish to a boil and continue boiling for one or two minutes in order to expel all CO_2. Now from a burette filled with decinormal sodium hydrate solution, run in this solution until we have the development of a faint but distinct pink in the boiling diluted bouillon which is not dissipated on further boiling.

It is more satisfactory to take burner from beneath the porcelain dish just before running in the N/10 solution, again boiling so soon as a pink color is obtained. Having obtained the light pink coloration we read off the number of c.c. or fractions of a c.c.of N /10 sodium hydrate solution added to produce the color. This number gives the acidity of the bouillon in percentage of N/1 acid solution.

Percent acid means that so many c.c. of N/1 acid added to 100 c.c. of the medium at the neutral point would give that percentage reaction. Thus 1 1/2 c.c. of N/1 HCl solution added to 100 c c. of medium at 0, would give us 1 1/2% of acidity or +1.5.

Percent alkaline means so many c.c. of N/1 sodium hydrate solution added to 100 c.c. of the medium at the neutral point. Thus a 1/2% alkaline medium would be one whose alkalinity would correspond to the addition of 1/2 c.c. of N/1 NaOH to 100 c.c. of the medium at 0. It is written −0.5.

If we took 100 c.c. of the medium and put it in a beaker and then ran in N/1 NaOH solution from a burette, it will be readily understood that if we had to add 3 1/2 c.c. of N/1 NaOH to obtain the pink color, it would show that the acidity of the 100 c.c. of medium, being tested, corresponded to 3.5 c.c. of N/1 acid solution, and that its acidity was equal to 3 1/2% of N/1 acid solution, or that its reaction was +3.5.

As N/1 NaOH solution is too corrosive for general use in a burette, and as 10 c.c. of medium is more convenient to work with than 100 c.c., we use a solution one-tenth the strength of the N/1 NaOH and we take only one-tenth of the 100 c.c. of medium. In this way it is the same from a standpoint of directly reading off our percentage reaction as if we had 100 c.c. of medium and used N/1 NaOH solution. The A. P. H. Association recommends 5 c.c. of the medium and the use of N/20 NaOH. As the N/10 NaOH is always at hand for titrating gastric juice, the N/10 is used instead.

Should it be found difficult to carry on the titration while boiling the end reaction may be fairly accurately determined in the cold. Deliver into a beaker from a pipette 10 c.c. of the bouillon and make up to 50 c.c. with distilled water and add 5 drops of 0.5% phenolphthalein solution. Then run in N/10 NaOH from a burette and continue to add the N/10 NaOH solution from the burette, drop by drop, until the addition of a drop fails to show any intensifying of the purplish violet color at the spot where it came in contact with the diluted bouillon in the beaker. This marks the end reaction. A reaction of about +0.7 in the cold gives a delicate pink with phenolphthalein as an indicator. Titration in the cold is not very satisfactory with gelatin and agar.

Having determined the percentage acidity of the 10 c.c. sample tested, we easily calculate the number of c.c. of N/1 NaOH solution required to be added to the 1000 c.c. of bouillon to obtain a reaction corresponding to the neutral point of phenolphthalein. It is more exact to take the average of two titrations.

As 100 c.c. of medium would require 3 1/2 c.c., 1000 c.c. would require 10 times as much, or 35 c.c. N/1 NaOH solution. Having measured out and added 35 c.c of the N/1 NaOH solution to the meat infusion, containing salt and peptone, we have a solution which is exactly neutral to phenolphthalein, or 0. It is usually considered that a reaction of about 1% acid is the optimum reaction for bacterial growth. Hence we should now add 1% of N/1 HCl solution to the medium. This would be accomplished by adding 10 c.c. of N/1 HCl solution to the 1000 c.c. of neutralized medium, and we would have a medium with a reaction of +1. If we desired a reaction of 1% alkalinity we would add an additional c.c. of N/1 NaOH solution to every 100 c.c. of the medium at 0, or 10 c.c. for the 1000 c.c. of medium. The reaction would then be −1.

As a matter of convenience, we usually determine the reaction of the medium, which is always more or less acid, and then add enough N/1 NaOH to reduce the acidity to the percentage we desire to set the medium, instead of neutralizing all the acidity present and then, in a second operation, restoring the acidity to the point desired.

Thus finding the acidity of the medium to be 3 1/2% and desiring to give it an acidity of 1%, we would add only 2 1/2 c.c. of N/1 NaOH to every 100 c.c. of medium, or 25 c.c. for the 1000 c.c. of medium. The reaction would then be found to be +1.

The neutral point of litmus is not a sharp one, but it corresponds rather closely with a reaction of +1.5 to phenolphthalein. The recommendations of the A. P. H. Association call for making the titration with the medium boiling. If the color of the end reaction at boiling-point be obtained, it will be found that when cool it deepens until it corresponds to the rich violet-pink of the end reaction in the cold or *vice versa.*

To summarize:

Take Peptone,	10 grams
Sodium chloride,	5 grams
50% meat infusion,	1000 c.c.

Dissolve the peptone and sodium chloride in the meat infusion and add enough N/1 NaOH to make the reaction +1.

Put the solution in the inner compartment of a rice cooker and bring to the boiling-point and maintain this temperature for twenty minutes. The calcium chloride or sodium chloride in the outer compartment of the rice cooker enables us to secure a boiling temperature for the contents of the inner compartment. Do not stir the bouillon that is being heated, as the pultaceous membranous mass of coagulated albumin makes filtration easy. Filter. The filter-paper in the funnel should be

thoroughly wet with water before pouring on the bouillon. This is to prevent clogging of the pores of the filter-paper. Make up the quantity of filtrate to 1000 c.c. by adding water.

If greater exactness is demanded than answers for ordinary clinical work, it is advisable to again titrate and again adjust the reaction or to simply record the exact reaction. It is more convenient to have a counterpoise to balance the inner compartment and then to add water to the medium until a kilo weight, in addition to the weight balancing the container, is just balanced. Then titrate, adjust the reaction (if so desired), and filter. Sterilize in the autoclave at 115° C. for fifteen minutes or in the Arnold on three successive days. The use of a balance is preferable in the preparation of bouillon, necessary in making gelatin and imperative in making agar media.

Bouillon Made from Liebig's Meat Extract.

Place in a mortar 3 grams of Liebig's extract, 10 grams of peptone and 5 grams of sodium chloride. Dissolve the whites of one or two eggs in 1000 c.c. of water. Then add this egg-white water, little by little, to the extract, peptone, and salt in the mortar until a brownish solution is obtained. Pour this into the inner compartment of a rice cooker; apply heat to the outer compartment containing the salt or calcium chloride solution, allow to come to a boil and to continue boiling for fifteen to twenty minutes. Do not stir. Place inner compartment on the scales and its counterpoise and a one-kilo weight on the other side. Add water until the two arms balance. Filter and sterilize.

The reaction of media made with Liebig's meat extract rarely exceeds +0.75 (from +0.6 to +0.9). Consequently for growing bacteria it is unnecessary to titrate and adjust reactions unless precision is demanded.

Sugar-free Bouillon.

Inoculate nutrient bouillon in a flask with the colon bacillus. Allow to incubate at 37° C. over night. Pour the contents into a sauce-pan and bring to a boil to kill the colon bacilli. Put about 15 grams of purified talc (Talcum purificatum, U. S. P.) in a mortar. Add the dead colon culture, stirring constantly. Then filter through filter-paper. It may be necessary to again pass the filtrate through the same filter until the sugar-free bouillon is perfectly clear.

For all ordinary purposes the very small amount of sugar in bouillon made from Liebig's meat extract may be neglected in determining gas production; so that under such conditions the various sugars could be added directly to the meat-extract bouillon.

Sugar Bouillons.

The sugar media ordinarily used for determining fermentation or gas production are those of glucose and lactose. In special work such carbohydrates as saccharose and maltose are used. The alcohol mannite is used in differentiating strains of dysentery bacilli.

To make, simply dissolve 1 or 2% of the sugar in sugar-free bouillon or that made from meat extract. Tube in Durham's or the ordinary fermentation tubes and sterilize in the autoclave at only about 5 pounds' pressure for 15 minutes, or in the Arnold. Ordinary peptone solution is a good substitute for sugar-free bouillon.

Too high a degree of heat may turn the sugar bouillon brownish. The nature of the sugar itself may further be affected by too high a temperature.

Calcium Carbonate Bouillon.

Where we wish to cultivate such organisms as streptococci and pneumococci in massive cultures we may add small fragments of marble (calcium carbonate) so that any inimical excess of acid may be neutralized. North used a glucose bouillon containing calcium carbonate in the production of massive cultures of B. bulgaricus.

Glycerine Bouillon.

Add 6% of glycerine to ordinary bouillon. It is chiefly used in the cultivation of tubercle bacilli.

Peptone Solution (Dunham's).

Dissolve 1% of Witte's peptone and 1/2% of sodium chloride in distilled water. Filter, tube, and sterilize. Peptone solution may be used as a base for sugar media instead of bouillon. It is the medium used in testing for indol production. This test is made by adding from 6 to 8 drops of concentrated H_2SO_4 to a twenty-four- to forty-eight-hour-old peptone culture of the organism to be tested. If the organism produces both indol and a nitroso body, we obtain a violet-pink coloration, "cholera red." If no pink color is produced on the addition of the sulphuric acid, add about 1 c.c. of an exceedingly dilute solution (1 : 10,000) of sodium nitrite.

It is very important in determining the "cholera red" reaction to know that the peptone used will give the reaction as it is not given by true cholera strains with certain samples of peptone.

For the Voges-Proskauer Reaction.—Fill fermentation tubes with a 2% glucose Dunham's peptone solution and sterilize. After inoculation with the organism to be tested incubate for three days. Then add 2 to 3 c.c. of strong caustic potash solution. The development of a pink color on exposure to the air is a positive reaction (the color of a weak eosin solution).

Hiss' Serum Water Medium.

Take one part of clear beef serum and add to it about 3 times it's bulk of water. Heat the mixture in the Arnold for 15 minutes to destroy any diastatic ferment which might be present. Color to a deep transparent blue with litmus solution and then add 1% of any of the various sugars used in fermentation tests. Sterilize in the Arnold by the fractional method.

Nutrient Agar.

In making agar medium it is preferable to use powdered agar, as this goes into solution more readily than the shredded agar. The reaction of agar is slightly

alkaline, so that if 1 1/2 to 2% of agar is added to nutrient bouillon having a reaction of +1 the finished product will be found to be about +0.8.

To make: Weight out 15 to 20 grams of powdered agar and place in a mortar. Make a paste by adding nutrient bouillon, little by little, and when a smooth even mixture is made, pour in into the inner compartment of a rice cooker and add the remainder of the 1000 c.c of bouillon. The use of the balance is preferable.

The outer compartment of the rice cooker should contain the 25% salt solution. Bring to boil, and the agar will be found to have entirely gone into solution after five to ten minutes of boiling.

Then, using a funnel which has been heated in boiling water and which contains a small pledget of absorbent cotton, we filter the agar, tube it, and sterilize it in the autoclave or Arnold. One and one-half percent agar can be readily filtered through filter-paper and gives a clearer medium.

By taking of meat extract 3 grams, peptone 10 grams, salt 5 grams, powdered agar 15 grams, the white of one egg and 1000 c.c. of water, making at first a paste of all the ingredients in a mortar, then gradually adding the remainder of the 1000 c.c. of water, putting in the rice cooker, bringing to a boil without stirring, allowing to boil fifteen minutes and then filtering through absorbent cotton placed between two layers of gauze in a hot funnel, we obtain a satisfactory medium, the reaction of which will be from +0.7 to +0.9. It is very important not to interfere with the pultaceous coagulum which forms on the surface of the boiling agar.

Where very exact adjustment of the reaction of the finished product is desirable the method of preparation of the Committee on Water Analysis of the American Public Health Association is to be preferred.

Dissolve 15 grams of agar in 500 c.c. of water in the inner compartment of the rice cooker previously described. After the agar is in solution (after 10 to 15 minutes boiling) remove the inner compartment, containing the 3% agar solution, and allow it to cool to about 55° C. Mix in the mortar, as described in the directions for making nutrient bouillon from Liebig's extract, 3 grams of Liebig's extract, 10 grams of peptone and 5 grams of sodium chloride in 500 c.c. of water containing the whites of one or two eggs. Heat this mixture to 50 to 55° C. and pour it into the agar solution, in the inner compartment, which has been cooled to about 55° C. Now titrate this mixture containing 500 c.c. of double strength agar and 500 c.c. of double strength peptone, meat extract and salt solution. The resulting 1000 c.c. gives 1 1/2% agar and 1% peptone solution. Having adjusted the reaction by the addition of the necessary amount of N/1 acid or alkali, we place the inner compartment in the outer one of the rice cooker, bring to a boil and filter through filter-paper which has been wetted with boiling water. The filtration can be carried out in the autoclave or in an Arnold sterilizer. Of course the ordinary filtering through gauze and cotton will answer where clearer media is not an object.

Glucose Agar.

Add the agar to 1 or 2% glucose bouillon and proceed as for ordinary agar. If preferred, the glucose agar can be made by rubbing up meat extract 3 grams, peptone

10 grams, salt 5 grams, glucose 10 grams and 15 grams of agar in 1000 c.c. of water containing the white of egg (one to two eggs), then boiling in the rice cooker and filtering.

Glycerine Agar.

Add the agar to 6% glycerine bouillon instead of nutrient bouillon, or the glycerine may be added to nutrient agar which has been melted. Glycerine agar with a reaction of 0 makes an excellent base for blood and serum media for use in culturing delicate pathogens.

Glycerine Agar Egg Medium.

Take the white and the yolk of one egg and mix thoroughly in a vessel kept between 45° and 55° C. with an equal amount of glycerine agar. Tube the medium, inspissate in a rice cooker as for serum tubes, and sterilize as for blood-serum tubes.

This makes an excellent medium for growing tubercle bacilli. As egg medium has a tendency to be dry, it is well to add 1 c.c. of glycerine bouillon to each slant before autoclaving.

Nutrient Gelatin.

Place in a mortar 3 grams of Liebig's extract, 10 grams of peptone and 5 grams of sodium chloride. Dissolve the whites of one or two eggs in 1000 c.c. of water. Then add this egg-white water, little by little, to the meat extract, peptone and salt, in the mortar, until a brownish solution is obtained. Pour this into the inner compartment of the rice cooker and bring the temperature up to 45° C. (This preliminary elevation of temperature is better carried out in some heated water in a pan, as the heating by means of the salt solution in the outer compartment of the rice cooker is difficult to control, so that a temperature approximating 70° C. might be obtained and the albumin of the white of egg coagulated. The temperature in the outer compartment might be approaching boiling before the contents of the inner compartment would show 45° C.) Now take about 120 grams of "gold label" or other good quality gelatin (12%) and crush it down in the meat extract egg-water solution in the inner compartment of the rice cooker.

The gelatin quickly goes into solution at 45° C. Gelatin being quite acid it will probably be found upon titration that the reaction is about +4%. N/1 NaOH solution is added to bring the reaction to about +1%. or 3 c.c. N/1 NaOH for each 100 c.c., provided the reaction were exactly +4%. The procedure is the same as for bouillon. The color reaction is not quite as distinct with gelatin as with bouillon.

Having neutralized and allowed to boil for fifteen minutes, we filter through filter-paper in a hot funnel. As it is very important that gelatin should be perfectly clear, it is better to filter through filter-paper than through cotton. The filter-paper should be very thoroughly wetted with very hot water before filtering gelatin or agar.

Tube the medium and sterilize, either in the Arnold on three successive days or in the autoclave at 8–10 pounds' pressure for ten minutes. The tubes should be cooled as quickly as possible in cold water after taking out of the sterilizer.

Agar Gelatin Medium (North).

Lean chopped beef or veal,	500 grams.
Agar,	10 grams.
Gelatin, Gold label,	20 grams.
Peptone, Witte's,	20 grams.
Sodium chloride,	5 grams.
Distilled water, *q.s.*,	1000 c.c.

Extract the chopped beef with 500 c.c. distilled water for 18 hours, strain through muslin and combine the ingredients in the usual way. Adjust the reaction to the neutral point, using phenolphthalein as indicator.

North states that this medium is excellent for streptococci, pneumococci and diphtheria bacilli because it is soft, moist, and can be used at 37° C.

It is claimed to be of special value for carrying stock cultures.

Litmus Milk.

Milk for media should be as fresh as possible. It should then be put in a 1000 c.c. Erlenmeyer flask, sterilized for fifteen minutes in the Arnold, and set over night in the refrigerator. The next morning the milk beneath the cream should be siphoned off. The short arm of the siphon should not reach the bottom of the flask so as to avoid the sediment. Add sufficient litmus solution to this milk to give a decided lilac tinge; tube and sterilize in the Arnold on three successive days.

Litmus milk which apparently is as satisfactory as the above as regards nutritive quality and cultural characteristics can be made from certain canned milks which have not been condensed or sweetened and which do not contain chemical preservatives. The "Natura" brand of milk is the one I have experimented with.

Litmus Solution.—A simple solution may be made by digesting the powdered cubes repeatedly with hot water, mixing the extracts, and, after allowing them to stand all night, decanting the solution from the inert sediment into a clean bottle.

In litmus solution so made, however, a red dye is also present while calcium and other salts are dissolved out. For bacteriological purposes a pure solution of the blue dye should be used. This is called "azolitmin." It is freely soluble in water but insoluble in alcohol.

It can be conveniently prepared as follows: Weigh out 2 ounces of powdered litmus; digest repeatedly with fresh quantities of hot water until all the coloring matter is dissolved out; allow to settle, and decant off the fluid from the insoluble powder. Add together the extracts, which should measure about a liter. Evaporate down the solution to a moderate bulk, then add a slight excess of acetic acid, so as to convert all carbonates present into acetates. Continue the evaporation, the later stages over a water bath, until the solution becomes pasty. Add 200 c.c. of alcohol, and mix thoroughly. The alcohol precipitates the blue coloring matter, while a red coloring matter, together with the alkaline acetate present, remains in

solution. Transfer to a filter. Wash out the dish with alcohol and add this to the filter. Wash the precipitate on the filter with alcohol. Dissolve the pure coloring matter remaining on the filter in warm distilled water and dilute to 500 c.c. Azolitmin solution prepared in this way is more sensitive than ordinary litmus solution.

Azolitmin in powder can be purchased from dealers in chemicals.

Potato Slants.

Take Irish potatoes and scrub throughly with a stiff brush. Then pare off generously all the outer portion. From the white interior cut out cylinders with a cork borer. These cylinders should be of 1/2 to 3/4 of an inch in diameter. Divide a cylinder by a diagonal cut. This gives a plug with a flat base, the other extremity being a slant. These potato plugs should be left in running water over night or washed with frequent changes of water. This prevents the blackening of the plug. Into a 1-in. test-tube drop a pledget of absorbent cotton well moistened with water. Then drop in the potato plug, base downward. Sterilize in the autoclave at 15 pounds for fifteen to twenty minutes, to insure sterility.

For glycerine potato, soak the plugs in 6% glycerine solution for about one hour. Then drop a pledget of absorbent cotton moistened with the same glycerine solution into the test-tubes and follow it with the potato plug. Sterilize in the autoclave.

Blood-serum.

The blood of cattle should be collected in large pans or pails at the abattoir. This vessel of blood should then be kept in the cold-storage room and the next morning the more or less clear serum will have been squeezed out from the clot. Collect this serum and keep in the ice chest for future use. If to be kept for a long time, it is advisable to add about 2% of chloroform to the serum in tightly corked flasks. This will not only keep the serum, but will eventually sterilize it.

To make Löffler's serum, take one part of glucose bouillon and three parts of blood-serum. Mix, tube, and coagulate the albumin in the inspissator or rice cooker, giving the tubes a proper slant before heating. Sterilize the following day in the autoclave as previously directed (7 lbs.) or in the Arnold on three successive days.

A Substitute for Ordinary Blood-serum.

Add from 10 to 15 c.c. of 1% glucose bouillon to the white and yolk of one egg, make a smooth mixture in a mortar and tube. Inspissate and sterilize as for ordinary serum slants. The morphology of the diphtheria bacilli and the luxuriance of growth is similar to that of cultures on Löffler's serum.

When this medium is to be used for culturing tubercle bacilli add about 1 c.c. of glycerine bouillon to each tube before final sterilization in the autoclave. The cotton plugs should be paraffined to prevent drying of the slants in the incubator. This medium seems to answer as a substitute for Dorset's egg medium. While glycerine bouillon favors growth of human tuberculosis, it is not so satisfactory for

bovine tuberculosis as plain glucose bouillon. This is better than the various white of egg substitutes usually recommended. (Pouring a little alcohol in the mortar and moistening the sides by tilting, then burning off the alcohol, in a measure sterilizes the mortar. If the egg is cracked open with a sterile knife, a medium can be prepared which will be sterile as the result of the two-hour inspissation in the rice cooker.) By covering the tube with a rubber cap or preferably, by heating the plugged end of the test-tube, quickly withdrawing the cotton plug and dipping the part of the plug which enters the tube into hot melted paraffin, then quickly reintroducing the plug, the contents of the tube will be prevented from drying out. This procedure is essential for growing tubercle bacilli. Dorset's egg medium for the cultivation of tubercle bacilli consists of the whole egg, which is emulsified as above, and heated at 70° C. for from four to five hours each day for two days. To provide moisture about 1 c.c. of sterile 6% glycerine solution is added to each slant.

Hydrocele, and Blood Agar.

To tubes of melted agar at 50° C, add from 1 to 3 c.c. of hydrocele or ascitic fluid, observing aseptic precautions. For blood agar the blood from a vein should be received into a sodium citrate salt solution to prevent coagulation, and added subsequently as for hydrocele fluid. Allow the agar to solidify as a slant, or as a poured plate.

Blood-streaked Agar.

Sterilize the lobe of the ear and puncture with a sterile needle. Collect the exuding blood on a large platinum loop and smear it over the surface of an agar slant. It is advisable to incubate over night as a test for sterility. Plates or slants of glycerine agar of neutral reaction smeared with blood give the best results when such delicate pathogens as pneumococci, streptococci, gonococci or meningococci are to be cultured.

Bile Media.

Secure ox bile from the abattoir or human bile from cases of gall-bladder drainage in hospitals. Put about 10 c.c. in each tube and sterilize. Some prefer to add 1% of peptone. Conradi's medium is ox bile containing 10% of glycerine and 2% of peptone. This is the medium for blood cultures in typhoid, etc.

The bile lactose medium now used in water analysis is made by adding 1% of lactose to ox bile and tubing in fermentation tubes. As a substitute for fresh bile one may use a 15 to 20% solution of a good quality of inspissated ox gall (Fel Bovis Purificatum). A liver bouillon made by using 500 grams of finely divided beef liver in 1000 c.c. of water with 1% peptone, and prepared as for meat infusion broth, is a good substitute for bile.

Rector's Bile Lactose Neutral Red Medium.

This is recommended in the isolation of the colon bacillus as superior to lactose litmus agar. It consists of 10% of dried ox bile, 1% of peptone, and 1 1/2% agar.

After the medium is filtered and tubed we add 1% of lactose and 1% of a 1–100 neutral red solution. Colon colonies have a distinct purplish red zone. Furthermore the bile inhibits the growth of many organisms which give pink colonies on lactose litmus agar. MacConkey's bile salt medium contains 1/2% of sodium taurocholate and is colored with neutral red.

Thalman's Medium for the Gonococcus.

Five hundred grams of lean, finely minced beef are placed in 1000 c.c. of distilled water and allowed to stand over night in an ice box. It is then filtered and the filtrate made up to 1000 c.c. with distilled water. To 100 c.c. of the beef juice add 1 1/2 grams of agar, and boil for 15 minutes. Then add 2 grams of glucose, and bring the reaction to plus 0.6 by addition of N/1NaOH. Tube, sterilize, slant, and incubate over night. No peptone or salt is required.

Plating Media for Fæces Work.

The media of Endo, Conradi-Drigalski and the lactose litmus agar medium are probably the most satisfactory of the numerous ones that have been proposed for plating out fæces. A convenient way of preparing any one or all of these, and which apparently gives media equal to that prepared according to the original formulæ, is as follows:

Liebig's extract,	5 grams.
Salt,	5 grams.
Pepton,	10 grams.
Agar,	30 grams.
Water to make	1000 c.c.

Prepare as for ordinary nutrient agar, with the difference that the reaction should be brought down to 0. Some prefer a reaction of +0.2.

A stiff agar (3%) is employed to check the diffusion of acid beyond the colony.

For Endo's Medium.

Keep this agar base in 100 c.c. quantities in Erlenmeyer flasks instead of test-tubes. (If more convenient smaller quantities may be put in the flask.) When needed for plating, melt a flask of this agar, and while liquid add to the 100 c.c. six drops of a saturated alcoholic solution of basic fuchsin, and then about twenty drops of a freshly prepared 10% solution of sodium sulphite. The sulphite solution decolorizes the intense red of the fuchsin to a light rose pink. This color fades to a light flesh or pale salmon color when cold. Now add 5 c.c. of a freshly prepared hot aqueous 20% solution of chemically pure lactose. If only occasionally using such media, tube in 20 c.c. quantities and add one drop of the basic fuchsin and four drops of the sodium sulphite solution and 1 c.c. of the hot freshly prepared lactose solution to a tube of the melted agar base just before pouring the plate. This medium contains 1% of lactose. Kendall prepares an Endo medium which only contains 1 1/2% of agar and with a reaction just alkaline to litmus (about plus 1.2%).

Colon bacilli show on this medium as vermilion colonies, which in about forty-eight hours have a metallic scum on them. Typhoid and dysentery colonies are grayish. Streptococci a deep red.

For Lactose Litmus Agar.

Color the agar base with litmus solution to a lilac color. Then add 5 c.c. of the hot freshly prepared lactose solution in distilled water. This may be tubed, putting 10 c.c. in each test-tube, or put in quantities of 50 or 100 c.c. in small Erlenmeyer flasks. It is then sterilized in the autoclave (10 pounds for fifteen minutes) or in the Arnold.

For Conradi-Drigalski Medium.

To 100 c.c. of lactose litmus agar add 1 c.c. of a solution of crystal violet (crystal violet 0.1 gram, distilled water 100 c.c.). The medium is then ready to put into plates. Colon colonies are pink. Typhoid and dysentery colonies, a bluish-gray.

Conradi's Brilliant Green Medium.

Take of Liebig's extract 20 grams (2%), peptone 10 grams (1%), agar 30 grams (3%)and water to 1000 c.c. This amount of meat extract should give about the proper acidity, +3. If not, the reaction should be adjusted to that point. Filter through cotton, tube 150 c.c. amounts into 250 c.c. Erlenmeyer flasks and sterilize.

Then add 1 c.c. of a 1 to 1000 aqueous solution of brilliant green (Höchst) and 1 c.c. of a 1% solution of picric acid to the flasks containing 150 c.c. of the melted agar. Sterilization after adding the dyes precipitates them and is unnecessary. Pour the finished medium into large Petri dishes and inoculate the surface with the fæces.

Brilliant green does not interfere with agglutination as does malachite green.

This medium is considered by some authorities the one of choice in isolating typhoid bacilli from fæces and urine.

The surface of the poured plates of Endo, Conradi-Drigalski, and the brilliant green media should be dried in the incubator before smearing with the fæces. For routine work I prefer Endo's medium followed by Russell's double sugar agar.

Selective Media for Cholera.

Dieudonne's medium rests on the ability of cholera to grow when alkali is present in such amounts as to inhibit the growth of other fæcal bacteria.

Take equal parts of defibrinated blood obtained at the slaughter house and normal NaOH solution. Mix 30 parts of this alkaline blood mixture with 70 parts of hot 3% nutrient agar. The poured plates should be left half open over night in the incubator otherwise even cholera will not grow on the plates.

Krumwiede has as a formula for his medium equal parts of whole egg and water, to which 50% water egg mixture is added an equal amount of 12 1/2% crystal sodium carbonate solution. This alkaline egg mixture is steamed for 20 minutes.

To prepare add 30 parts of this alkaline egg mixture to 70 parts of meat extract free 3% agar. (No meat extract; only peptone and salt.) The cholera colony has a hazy look, like a little wad of absorbent cotton sticking to the surface with a metallic luster halo.

Russell's Double Sugar Agar.

A fairly stiff agar (2 to 3%) with a reaction of about plus 0.7 is colored with litmus solution to produce a distinct purple violet color. It may be necessary to add more alkali. To this litmus tinted agar is added 1% of lactose and 0.1% of glucose and the medium as thus prepared is tubed and slanted. Sterilization should be carried on in the Arnold, on two successive days, as the autoclave temperatures tend to break up the sugars.

On these slants typhoid shows a delicate growth on the violet slant with a deep pink in the butt of the tube. The paratyphoids show gas bubbles in a pink butt with a violet slant.

The colon bacillus turns both slant and butt a deep pink and the butt is filled with gas bubbles. To inoculate this medium we take material from a suspicious colony grown on Endo and smear the material on the slant; then with the same platinum needle we stab into the butt.

Culture Media for Protozoa.

Medium of Musgrave and Clegg.

Dissolve in 1000 c.c. of water 0.3 to 0.5 gram Liebig's extract and 0.3 to 0.5 gram of common salt. If desired for plating add 2 to 3% of agar.

A very satisfactory substitute is ordinary nutrient bouillon diluted one to ten.

Medium of Smith.

Glucose 1.0 gram; Peptone 1.0 gram; NaCl 0.2; Aqua destill. 1000.0; Na_2CO_3 0.3. Agar q. s. is added for solid medium.

Medium of Castellani.

This is an aqueous medium containing 1% of lactose and 10% of agg albumin. This may replace water of condensation in an agar slant.

Novy MacNeal Medium.

Cover 125 grams of chopped up beef with 1000 c.c. of water and place over night in the refrigerator. Strain and add 20 grams of peptone, 5 grams salt, 10 c.c. of normal sodium carbonate solution and 20 to 25 grams agar. Prepare as for nutrient agar and sterilize. To 1 part of this one-quarter strength meat infusion nutrient agar, when melted and cooled down to 60° C., add twice its volume of de-

fibrinated rabbit's blood. This medium is the standard one for the culture of certain trypanosomes and other protozoa. Under the designation N.N.N. medium (Nicolle Novy MacNeal) Nicolle has modified the medium so that there is only salt and agar in the base to which the blood is added instead of one containing meat extract and peptone. It is the Hb which seems essential in the culture of various protozoa. Rogers used citrated salt solution, which was slightly acidified with citric acid, in his culturing of Leishmania from the splenic blood of cases of kala azar. Incubation at 22° C.

Row's Hæmoglobinized Saline Medium.

Take 10 c.c. blood from rabbit's heart or arm vein of man, defibrinate the blood and then add 10 volumes of distilled water to lake the cells (liberation of Hb). One volume of this laked blood solution is added to two volumes of sterile 1.2% salt solution.

Culture Media for Treponemata.

I. Noguchi formerly first inoculated material containing treponemata into the testicle of rabbits, obtaining by this procedure a pure culture, after a few transfers to the testicles of other rabbits. He now grows the organism directly from serum from a chancre. Test-tubes 2 by 20 cm. are filled with 15 c.c. of a medium consisting of 2 parts of 2% slightly alkaline agar to which when melted and cooled down to 50° C. is added 1 part of ascitic or hydrocele fluid. At the bottom of the medium in the tube is placed a fragment of fresh sterile tissue, preferably a piece of rabbit's kidney or testicle. After the medium solidifies a layer of sterile paraffin oil is run in so that it covers the solid medium to a depth of 3 cm. The material is inoculated at the bottom of the tube with a capillary pipette. Incubation at 37° C. is carried on for two weeks. The tissue acts by removing any oxygen that may be present in the depths of the medium. Anaerobiosis is a necessary condition. Many specimens of ascitic fluid are unsuited.

II. **Serum Agar of Muhlens and Hofmann.**—Fill sterile test-tubes one-third full with horse serum. This is sterilized on three successive days at 55° C. Then add an equal amount of a 3% agar containing 0.5% glucose which has been melted down and cooled to 50° C. The mixed serum agar is then kept at 55° C. for two hours. Such tubes are inoculated as for ascitic agar rabbit tissue media and incubated under anaerobic conditions, preferably in a flask from which the air has been exhausted and the remaining oxygen absorbed as shown in the anaerobic bottle described and illustrated in Fig. 7.

Wellman's Placental Agar.

Fresh human placenta is thoroughly ground up in a meat chopper, after first washing out the blood by running sterile salt solution through the attached vessels. To each kilo of the macerated placental tissue is added 1 liter of distilled water. This mixture is allowed to infuse for forty-eight hours at refrigerator temperature, after which it is passed through a No. N Berkefeld which has been previously tested

and found to hold back ordinary bacteria. The first half-hour's filtrate is usually found to be perfectly sterile. To facilitate this filtration the cylinder of the filter is filled with fine, clean, sterile sand until the candle is completely covered. The filtrate is either tubed or added to 2% sterile, previously melted agar at 40° to 41° C., mixed, and slanted. No titration or other preparation is necessary, except that the medium is placed at a temperature of 40° C. for two days to inactivate the complement, as suggested by Bass in the use of human blood cultures. Fresh human placenta contains over 30% of the hydrolytic products of protein digestion, and will therefore secure growths of strictly parasitic or feebly vegetative bacteria, and possibly protozoa, that are grown with great difficulty or not at all on ordinary media. For instance, the acid-fast organisms from bits of leprous tissue, either of human or rat origin, grow on this medium so readily that microscopic growth can be discerned in from five to seven days. From human tuberculous glands, urine, or cerebrospinal fluid the same method will give a growth of *B. tuberculosis* that can be distinguished in from seventy-two hours to a few days.

CHAPTER III.

STAINING METHODS.

In order to study a bacterial or blood specimen the first essential is a properly prepared film; the matter of staining is of less importance. The slide or cover-glass, after cleaning with soap and water or by special solutions, should be polished with a piece of old linen. If a glass surface is free of grease a loopful of water will smear out evenly and over the entire surface. The only quick practical way to make the slide or cover-glass grease free is to burn the surface for a moment in a Bunsen or alcohol flame. The cover-glass must not be warped. To make a preparation, apply a small loopful of distilled water on the slide or cover-glass and, touching a colony with a platinum needle, stir the transferred culture into the loopful (not drop) of water. The mistake is almost invariably made of taking up too much bacterial growth. Fluid cultures do not need dilution. Smearing the mixture over a large part of the cover-glass or over an equal area of a slide, it is allowed to dry. If very little water is used, the preparation dries readily. Otherwise it can be dried in the fingers high over a flame. As soon as dry, the cover-glass should be passed three times through the flame, film side up, to fix the preparation. Slides may be fixed by passing them five times through the flame, but the method by burning alcohol recommended for fixing blood-films gives more satisfactory bacterial fixation. For routine work the stain recommended is a dilute carbol fuchsin. Drop about 5 to 10 drops of water on the cover-glass, then add one drop of carbol fuchsin. Allow the dilute stain to act from one to two minutes, then wash in water, dry between small squares of filter-paper (4×4 inches), and mount in balsam or the oil used for the 1/12-inch immersion objective.

By far the best mounting medium is liquid petrolatum. This not only has the advantage of always being of proper consistence for mounts, as opposed to Canada balsam, which must frequently be made thinner with xylol, but it is less sticky and does not develop the acidity which causes balsam mounts of Romanowsky stains to fade. Furthermore, it has superior optical qualities. It is also applicable for mounting small insects and sporangia of moulds. For permanent preparations the

border of the cover-glass should be sealed with gold size or some other cement. Some prefer to mount directly in water without preliminary drying. It is good practice to make a rule to always keep the smeared side of the preparations up—never allowing it to be reversed. By this simple rule, preparations can be carried through the most complicated staining methods without the necessity of scratching the cover-glass, etc., to see which is the film side. In grasping a cover-glass with a Cornet or Stewart forceps, be sure that the tips are well by the margin of the glass, otherwise the stain will drain off. In staining with slides, the grease pencil and the glass tubing, as recommednded under Blood Smears, will be found useful. The dilute carbol fuchsin and Löffler's methylene blue are probably the best routine stains. As a rule better preparations are obtained with dilute stains than with more concentrated ones.

Löffler's Alkaline Methylene Blue.—Saturated alcoholic solution of methylene blue, 30 c.c.; one to ten thousand caustic potash solution, 100 c.c. (Two drops of a 10% solution KOH in 100 c.c. of water makes a 1 : 10,000 solution.)

Carbol Fuchsin (Ziehl-Neelsen).—Saturated alcoholic solution basic fuchsin, 10 c.c.; 5% aqueous solution carbolic acid, 100 c.c.

Gram's Method.—The most important staining method in bacteriological technic and the one so rarely giving satisfactory results to the inexperienced is Gram's stain. In using this method, the following points must be kept in mind:

1. Laboratory cultures (subcultures) which have been carried over for years frequently lose their Gram characteristics.

2. Cultures which are several days old or dead or degenerated do not stain characteristically.

3. The aniline gentian violet deteriorates when exposed to light in two or three days—it should be kept in the dark. It should have a rich, creamy, violet appearance.

4. The iodine solution deteriorates and becomes light in color. It should be of a rich port-wine color.

5. The decolorizing with 95% alcohol should stop as soon as no more violet stain streams out. This is best observed over a white background, washing at intervals. Do not confuse stain on forceps for that on preparation.

6. The preparation should be thin and evenly spread. Some prefer carbol gentian violet to aniline gentian violet. (Saturated alcoholic solution of gentian violet, 1 part; 5% aqueous solution of carbolic acid, 10 parts.) This tends to overstain.

The formula for aniline gentian violet is 1 part of saturated alcoholic solution gentian violet and 3 parts of aniline oil water (made by adding 2 c.c. aniline oil to 100 c.c. distilled water, shaking violently for three to five minutes and then filtering several times to get rid of the objectionable oil droplets which, in a Gram-stained preparation, show as confusing black dots).

The following stock solutions of Weigert are recommended:

No. 1.		No. 2.	
Gentian violet,	2 grams.	Gentian violet,	2 grams.
Aniline oil,	9 c.c.	Distilled water,	100 c.c.
Alcohol (95%),	33 c.c.		

These stock solutions keep indefinitely. Mix 1 c.c. of No. 1 with 9 c.c. of No. 2. Filter. This keeps about two weeks and is the solution to pour on the preparation. It may be kept on from two to five minutes. Some hasten the staining by steaming as for tubercle bacilli. Next wash the preparation with water and flood the cover-glass with Gram's iodine solution. Some bacteriologists simply pour off excess of aniline gentian violet and immediately drop on the iodine solution. It is well to repeat the application of the iodine solution a second time. The iodine solution is left on one minute or until the preparation has a coffee-grounds color.

Gram's Iodine Solution.

Iodine,	1 gram.
Potassium iodide,	2 grams.
Distilled water,	300 c.c.

After washing off the excess of iodine solution at the tap, drop on 95% alcohol and decolorize until no more violet color streams out. Now wash again and counter-stain either with the dilute carbol fuchsin or with a saturated aqueous solution of Bismark brown.

The Gram-positive bacteria are stained a deep violet.

In staining smears of pus for gonococci or other Gram-negative bacteria it is best to first stain with the gentian-violet solution for two to five minutes. Then wash and examine the preparation mounted in water. The organisms stand out prominently. After noting the presence of the cocci treat the smear with the Gram solution and proceed as in the usual Gram staining technic.

Stained by Gram's Method.	*Not Stained by Gram's Method.*
S. pyogenes aureus.	Meningococcus.
S. pyogenes albus.	M. catarrhalis.
S. pyogenes.	M. melitensis.
M. tetragenus.	B. typhosus.
Pneumococcus.	B. coli communis.
Anthrax bacillus.	B. dysenteriæ (Shiga).
Tubercle bacillus.	Sp. choleræ asiaticæ.
Lepra bacillus.	B. pyocyaneus.
Tetanus bacillus.	B. mallei.
Diphtheria bacillus.	B. pneumoniæ (Friedländer).
B. aerogenes capsulatus.	B. proteus.
Oidium albicans.	B. of influenza.
Mycelium of actinomyces.	B. of bubonic plague.
Saccharomyces.	B. of chancroid.
Hofman's bacillus.	B. of Koch-Weeks.
B. xerosis.	Gonococcus.

Practically all pathogenic cocci are Gram-positive, except the Gonococcus, the Meningococcus, the M. catarrhalis, and the M. melitensis.

Practically all pathogenic bacilli are Gram-negative, except the spore-bearing ones (exception B. malig. œdemat.), the acid-fast ones and diphtheria and diphtheroid organisms.

The bacillus of glanders is Gram-negative.

Method for Staining Acid-fast Bacilli.—1. Carbol fuchsin, with gentle steaming for three to five minutes or in the cold for fifteen minutes.

2. Wash in water.

3. Decolorize in 95% alcohol containing 3% of hydrochloric acid (acid alcohol), until only a suggestion of pink remains—almost white.

4. Wash in water.

5. Counterstain in saturated aqueous solution of methylene blue or with Löffler's methylene blue.

6. Wash, dry, and mount.

The steaming of the slides with carbol fuchsin is most conveniently carried out by resting the slides on a piece of glass tubing bent into a V or U shape.

A method in which the organisms or granules which stain by the Gram method, and to which so much importance is attributed by Much, may be stained, as well as those retaining acid-fast properties, has been proposed by Fontes. The method is to stain the preparation with carbol fuchsin, decolorize with acid alcohol, then carry through the various steps of the Gram method, counterstaining however, with Bismark brown. Fontes in his method used 1 part of absolute alcohol and 2 parts of acetic acid as the decolorizing agent. I have obtained, however, just as satisfactory results with the acid alcohol. By this method the acid-fast tubercle bacilli show as red rods dotted with violet granules. Those which do not fully retain acid-fast properties show as zigzag violet lines.

Herman's Stain for Tubercle Bacilli.—It has been claimed that this stain gives better satisfaction than the Ziehl-Neelsen. It consists of two solutions: (1) ammonium carbonate in distilled water, 1%; (2) crystal violet (methyl violet 6B) in 95% ethyl alcohol, 3%. The two solutions are kept in separate bottles and, for staining, 1 part of (2) is mixed with 3 parts of (1). The sections are placed on a cover-glass, the water evaporated, and about seven drops of the staining mixture are placed on the specimen and allowed to steam for one minute over a water-bath. Place for a few seconds in 10% nitric acid and then in 95% alcohol to decolorize. Mount without a counterstain or use eosin 1% or a very dilute fuchsin. The organisms are purple. This staining method may be applied to smears of concentrated or unconcentrated sputum in the same manner as for sections of tissue.

Smith's formol fuchsin:

Saturated alcoholic solution basic fuchsin,	10 c.c.
Methyl alcohol,	10 c.c.
Formalin,	10 c.c.
Distilled water to make	100 c.c.

This gives a very sharp differentiation of bacteria and nuclear structures. It has a purplish tinge. Fixation by heat gives the best staining. Allow the stain to act for two to ten minutes. It should not be used until after standing twenty-four hours, and after standing about two weeks it appears to lose its sharp staining power.

Archibald's Stain.—This is an excellent bacterial stain and has been highly recommended by Blue and McCoy in plague work.

Solution No. 1.		*Solution No. 2.*	
Thionin,	0.5	Methylene blue,	0.5
Phenol crys.,	2.5	Phenol crys.,	2.5
Formalin,	1.0	Formalin,	1.0
Water,	100.0	Water,	100.0

Dissolve for twenty-four hours. Mix equal parts and filter. Stain smears fixed by heat or otherwise for ten seconds.

Nicolle's Carbol Thionin.

Sat. sol. thionin in 50% alcohol,	10 c.c.
Carbolic acid solution (2%),	100 c.c.

Pappenheim's Stain.—Take a very small portion of methylene green on the point of a penknife and shake it into a test-tube. Then take up twice as much pyronin and deposit it in the same test-tube. Fill the test-tube one-half full with water and the solution should have a distinct reddish-violet color. A drop on a piece of filter paper shows a violet center and peripheral green ring. The solution should be fresh. Stain from two to five minutes. Differentiate with a little resorcin on a penknife point dissolved in one-quarter of a test-tube full of alcohol. Dehydrate, clear and mount. Polymorphonuclear nuclei stain greenish; nuclei of mononuclears and plasma cells from bluish-red to dull violet. Cytoplasm of lymphocytes and plasma cells purplish-red. Bacteria red.

Romanowsky Stains.—See under section on Blood. For mounting specimens showing chromatin staining, as malarial parasites, trypanosomes, intestinal flagellates etc., liquid petrolatum is to be highly recommended. The chromatin staining lasts without any fading for at least two years. The acidity of balsam causes rapid fading of the chromatin.

Neisser's Stain for Diphtheria Bacilli.

Solution No. 1.		*Solution No. 2.*	
Methylene blue,	0.1 gram.	Bismark brown,	0.2
Alcohol,	2 c.c.	Water (boiling),	100 c.c.
Glacial acetic acid,	5 c.c.	Dissolve the stain in the boiling water and filter.	
Distilled water,	95 c.c.		
Dissolve the methylene blue in the alcohol and add it to the acetic acid water mixture. Filter.			

To stain: Fix the preparation. Pour on the dilute acetic acid methylene blue solution and allow to act from thirty to sixty seconds. Wash. Then pour on the Bismark-brown solution, and after thirty seconds wash off with water. Dry and mount. The bodies of the bacilli are brown with dark blue dots at either end.

Neisser recommends only five seconds as the time of application of each solution. He also recommends that the culture be only nine to eighteen hours old and that the temperature of the incubator shall not exceed 36° C. Incubation at 37° C. gives satisfactory results.

Ponder's Stain For Diphtheria Bacilli.

Toluidin blue (Grubler),	0.02 gram.
Glacial acetic acid,	1 c.c.
Absolute alcohol,	2 c.c.
Distilled water to	100 c.c.

The film is made on a cover-glass and fixed in the usual way. A small quantity of the stain is spread on the film and the cover-glass is turned over and mounted as a hanging-drop preparation. The metachromatic granules of the diphtheria bacilli stain with striking intensity. With diphtheroids, the more intense staining sharply differentiates from ordinary cocci and bacilli, which show in the preparation only as faint light blue bodies. It is a most excellent stain for bringing out the ascopores of yeasts. In my opinion the stain is more valuable than the Neisser method.

Capsule Staining.—The best method for studying bacteria, as to presence of capsules, is in the hanging drop, with the greater part of the light shut off by the diaphragm.

In material where capsules are well developed, as in pneumonic sputum, the Gram method of staining brings out the capsule perfectly. This is of diagnostic value, as the more or less nonpathogenic pneumococci common about the mouth do not seem to show a capsule when stained in this way. The India ink method of staining gives good results for capsules.

The most beautiful method of staining capsules is the latest one proposed by Muir.

1. Prepare thin film, dry and stain in carbol fuchsin one-half minute; the preparation being gently heated (steamed).
2. Wash slightly in 95% alcohol, then wash well afterward in water.
3. Flood preparation in mordant for five to ten seconds.

Mordant.—Sat. aqueous sol. mercuric chloride,	2 parts
Tannic acid (20% aqueous sol.),	2 parts
Sat. aqueous sol. potash alum,	5 parts

4. Wash in water thoroughly.
5. Treat with 95% alcohol for one minute. (The preparation should have a pale red color.)
6. Wash well in water.

7. Counterstain with methylene blue one-half minute.
8. Dehydrate in alcohol. Clear in xylol and mount. (May simply dry specimen with filter-paper.)

Rosenow's Capsule Stain.—Make a very thin smear of the pathological material and when nearly dry cover the preparation for ten to twenty seconds with 10% tannic acid solution. Wash in water and blot. Stain with aniline gentian violet by gently steaming for one-half to one minute. Wash in water. Apply Gram's iodine solution for one-half to one minute. Decolorize in 95% alcohol and then stain with alcoholic solution of eosin. Wash in water, dry and mount.

Flagella Staining.—Inoculate a tube of sterile water (gently) in upper part, with just enough of an eighteen to twenty-four-hour-old agar culture to produce faint turbidity. Incubate for two hours at 37° C. From the upper part of culture take a loopful and deposit it on a cover-glass. Dry in thermostat for one to five hours or over night. Use perfectly clean cover-glasses. To stain by

Muir's Modified Pitfield Method.

1. Flood specimen with mordant. Steam gently one minute.
   Mordant.—Tannic acid (10% aqueous solution), 10 c.c.
   Sat. aq. sol. mercuric chloride, 5 c.c.
   Sat. aq. sol. alum, 5 c.c.
   Carbol fuchsin, 5 c.c.
   Allow precipitate to settle or centrifuge. Keeps only one week.
2. Wash well in water for two minutes.
3. Dry carefully—preferably in incubator.
4. Pour on stain. Steam gently one minute.
   Stain.—Sat. aq. sol. alum, 10 c.c.
   Sal. alc. sol. gentian violet, 2 c.c.
   (May use carbol fuchsin instead of gentian violet.)
   Stain only keeps two days.
5. Wash well in water. Dry and mount.

Zettnow's Flagella Staining Method.

Solution I.—Dissolve 2 grams of tartar emetic in 40 c.c. water.

Solution II.—Dissolve 10 grams tannin in 200 c.c. water. To the 200 c.c. solution II, warmed to 50 or 60° C., add 30 c.c. of the tartar emetic solution. The turbidity of the mordant should entirely clear up on heating. The mordant should keep for months when a small crystal of thymol is added to it.

Next dissolve 1 gram silver sulphate in 250 c.c. distilled water. Of this solution take 50 c.c. and add to it drop by drop ethylamine (this comes in a 33% solution) until the yellowish-brown precipitate which forms at first is entirely dissolved and the fluid is entirely clear. It requires only a few drops. The bacterial preparations prepared as described above are floated in a little mordant contained in a Petri dish

which is heated over a water bath for five to seven minutes. Take the dish containing the preparation off the water bath and as soon as it becomes slightly opalescent as the result of cooling remove the cover-glass preparation and wash thoroughly in water. Then heat a few drops of the ethylamine silver solution upon the mordanted cover preparation until it just steams and the margin appears black. Next wash thoroughly in water and mount. This gives the most satisfactory results of any method I have ever experimented with.

Spore Staining.—The most satisfactory spore staining method is really the negative staining of the spore obtained when a bacterial preparation is stained by dilute carbol fuchsin or Löffler's methylene blue. The spore appears as a highly refractile piece of glass in a colored frame.

The acid-fast method, as for tubercle bacilli, gives good results. The decolorizing, however, must be lightly done, otherwise the spore will lose its red stain.

Möller's Method.—Fix films and then treat with chloroform for one or two minutes. Wash thoroughly and treat with a 5% solution chromic acid for one minute. Wash in water and then stain as for acid-fast organisms with carbol fuchsin. Use a 1% sulphuric acid solution instead of the 3% acid alcohol.

Agar Jelly Staining Method of H. C. Ross.

Very clear 1 1/2% solution of agar is colored with Unna's polychrome methylene blue, Giemsa's solution, thionin or Gram's solution of iodine. Very thin smears of blood, fæces or gastric content sediment are made and either fixed lightly in the flame or air dried. A drop of the melted colored agar solution is placed on the smeared cover-glass and this is mounted immediately on a clean slide. The preparation is ready for examination in about two minutes.

The Staining of Protozoa.

Unless staining albuminous material it is well to add a little blood-serum or white of egg to the preparation—about one loopful to a smear. The serum or white of egg is best preserved by the addition of 2% chloroform and kept tightly corked.

Giemsa's Method.—Fix moist smears with a fixative made by adding 1 part of 95% alcohol to 2 parts of saturated aqueous solution of bichloride of mercury. Keep in this solution twelve hours. Now wash for a few seconds in water and then for about five minutes with a dilute Lugol's solution (KI, 2 gm.; Lugol's solution, 3 c.c.; Aqua, 100 c.c.). Now wash in water and then in a 0.5% solution of sodium thiosulphate to remove the iodine which was used to remove the mercury.

Wash in water five minutes, then stain with Giemsa's stain as used in blood work for one to ten hours. Wash and mount.

Vital Staining of Protozoa with Neutral Red Solution.—As a stock solution one uses a 0.5% aqueous solution of neutral red.

The drop of salt solution or water on the slide should be tinged a light violet-rose color with a fraction of a loopful and the fæces or other material emulsified in this.

Protozoa take a rose-pink color with a distinct differentiation between endoplasm and ectoplasm.

Should the fæces be quite alkaline the neutral red will be decomposed with the formation of bilirubin-like crystals.

The Giemsa formalin method described under Blood Work is of value in certain cases.

Highly to be recommended for the staining of protozoa, whether in smears or in sections, is the *Panoptic method.*

1. Wright's or Leishman's stain for one minute.

2. Dilute with water and allow dilute stain to act for three to ten minutes. Wash in water and then

3. Pour on dilute Giemsa's stain. Allow to stain from thirty minutes to twenty-four hours. Differentiate with 1 : 1000 acetic acid solution until blue stain just shows commencing diffusion into the acetic acid. Then wash in water, 95% alcohol, absolute alcohol and treat with xylol and mount in liquid petrolatum.

With preparations other than blood smears, as sections, it is better to go from 95% alcohol to oil of origanum, then mount.

Owing to the great value of a sharp nuclear picture in differentiating amœbæ it is of great importance to use some iron hæmatoxylin method. That of *Weigert* is given in the appendix.

Fix moist smears, film surface down, in Zenker's fluid for five to ten minutes. Wash in water, treat with Gram's solution and wash with 70% alcohol until all the yellow color is discharged. Wash in water. Then stain with *Mallory's phosphotungstic hæmatoxylin* for one-half hour. Wash clear and mount. See appendix.

Mallory's Differential Stain for Amœbæ.—Staining in saturated aqueous solution thionin for from three to five minutes. Next differentiate in 2% aqueous solution oxalic acid for one-half to one minute. Then wash in water, clear and mount. Nuclei of amœbæ are stained a brownish red.

CHAPTER IV.

STUDY AND IDENTIFICATION OF BACTERIA—GENERAL CONSIDERATIONS.

In order to study bacteria it is necessary to isolate them in pure culture. This may be accomplished by taking one or more loopfuls of the material and mixing it in a tube of melted agar or gelatin. From this first tube one or more loopfuls are transferred to a second tube of melted agar or gelatin, and from this a third transfer is made, thereby giving us tubes in which the distribution of the bacteria is one or more hundred times less in the second than in the first tube, and equally more dilute in the third than in the second. When we pour the contents of the tubes into Petri dishes we would have the bacterial colonies on the first plate so thick that it would be impossible to pick up a single colony with a platinum needle without touching an adjacent one. On the second plate the distribution might be such that we should have discrete, well separated colonies, material from which could be taken up on the point of the needle or loop without touching any other colony. If the second plate did not meet these requirements, the third would.

In clinical bacteriology we work almost entirely with organisms preferring blood-heat temperature, hence it is necessary to use agar or blood-serum as standard media for the obtaining of isolated colonies. Gelatin is of little value for this purpose in medical work. In using agar it will be remembered that it solidifies at a temperature slightly below 40° C. and does not melt again until it is subjected to a temperature practically that of boiling. Again, if the temperature of the media exceeds 44° C. it may affect injuriously the organisms we wish to study. Consequently it requires careful attention and quick work to inoculate the tubes, mix, transfer and pour into plates within the limits of a temperature which injures the organisms, and one which brings about the solidification of the agar.

Again, we not only have colonies developing from organisms which have been fixed at the surface as the agar solidified in the plate, but more numerous ones developing from bacteria caught in the depths

of the media. Therefore we have superficial and deep colonies. Except to the person of great experience, all deep colonies look alike and there is at times great difficulty in deciding whether a colony is deep or superficial. It is in the matter of trying to obtain information from the differences in deep colonies that the greatest difficulties in the study

FIG. 8.—Petri agar plate. Made by spreading scrapings from the mouth over sterilized nutrient agar; after forty-eight hours in the thermostat the light "colonies" develop. Streaked plate. (*Delafield and Prudden.*)

of bacteriology arise. By using the method of simply stroking plates along five or six parallel lines from one side of the plate to the other with a bent glass rod, platinum loop, or a small cotton swab, we obtain colonies which are well separated and which are entirely superficial.

The material as pus, fæces, throat membrane, etc., should be evenly distributed in a tube of sterile water or bouillon; the swab which was

originally used for obtaining the material being then pressed against the sides of the test-tube to express excess of fluid and then stroked gently over successive lines on one plate. Or, if the organisms be very abundant, over a second plate without recharging it from the inoculated tube.

According to my experience a very satisfactory method is to take a loopful from the bouillon tube suspension of the pus or fæces and deposit the fluid in the platinum loop on the left half of the poured plate then, without recharging the loop, we touch the right half of the plate. Now taking a bent glass rod from a jar of 95% alcohol we flame it and to cool the same we press the bent portion into the middle of the plate. This also divides the surface of the plate into two portions. Then rubbing the bent rod over the smaller amount of the material on the right side we carry it over the entire right side. Then go to the loopful deposited on the left side with the rod and rub it over this side. For urine, deposit one drop on one side and 5 drops on the other. A smear from pus, sputum, urine or throat culture should always be made first in order to get an idea as to the degree of dilution which is necessitated before plating out.

To obtain isolated colonies on blood-serum or blood-streaked agar, which can be touched and by transfer obtained in pure culture, we simply smear the material on a slant of either medium. Then, without sterilizing the loop, we smear it thoroughly over a second slant, and so on to a third, or possibly a fourth or fifth.

At present the classification of the bacteria is very unsatisfactory from a scientific standpoint. The nomenclature abounds in instances where three or four terms are used in naming a single bacterium, instead of the single generic name and single specific one as is used in zoölogical nomenclature. This matter of nomenclature is a subordinate factor in the confusion when we begin to investigate and find that different names have been applied to apparently the same organism. The slightest variation in morphological, locomotor, or biological characteristics seems to be considered sufficient by many observers to justify the description of a new species, and, of course, the giving of a new name. Many of these names which are now retained were applied prior to the epoch-making introduction of gelatin media by Koch (1881) and consequently at a time when the isolation of organisms in pure culture was a matter of extreme difficulty and uncertainty. One of the first facts noted by the student in taking up bacteriology is the difficulty in determining motility; this property should always be tested on young cultures in bouillon. In Brownian movement there is a sort of scintillating movement, but the bacterium does not move

from that part of the field. In current movement all the bacteria swarm in the same direction, going very fast at times, and then more slowly. If in great doubt, the mounting of the organisms in a 2% solution of carbolic acid will stop movement if it be true functional motility, while Brownian and current movement are not interfered with. In true motility bacteria move in opposite and in all directions,

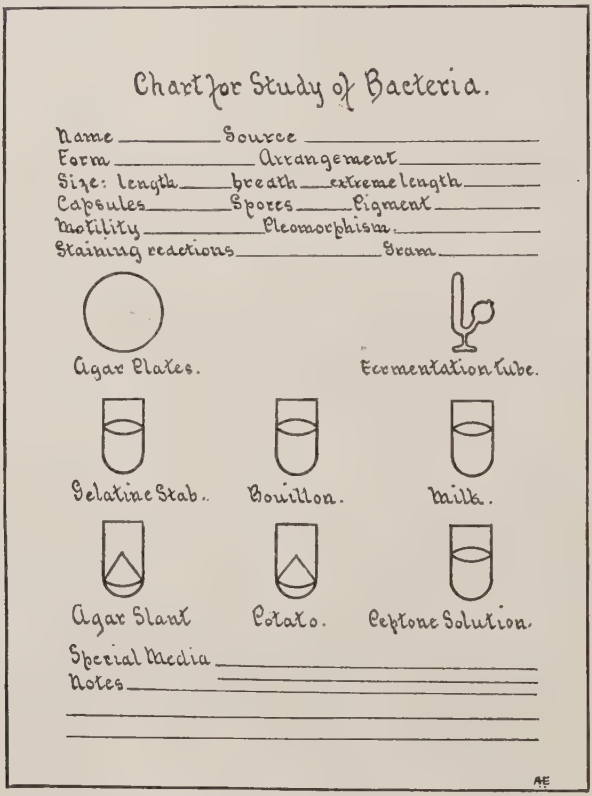

FIG. 9.—Chart in use at the U. S. Naval Medical School.

and move away from the place where first observed unless degenerated or dead.

At times we judge of motility by the presence of this characteristic in a few of the organisms seen in the microscopic field, the vast majority of the bacteria not showing motility. A source of error can be present when the bacteria are emulsified in a drop of water which might contain motile bacteria.

Reaction of media is of the greatest importance in causing variation in the functions of bacteria, and is one which has until recently been almost entirely neglected. In describing an organism at the present time it is always necessary to note the reaction of the media, the temperature at which cultivation took place, and the age of the culture when examined.

In the following keys the term bacterium has been used as a general designation for all schizomycetes. Migula calls motile rod-shaped organisms bacilli, and nonmotile ones bacteria. Lehmann and Neumann call spore-bearing organisms bacilli, and nonspore-bearing ones bacteria.

The B. typhosus is very motile and does not possess spores. According to Migula, it would be the Bacillus typhosus; according to Lehmann and Neumann, the Bacterium typhosum. The B. anthracis has spores and is nonmotile. Hence it would be Bacterium anthracis, according to Migula, and Bacillus anthracis, according to Lehmann and Neumann.

In the use of the keys at the head of each group of organisms it will be observed that the primary separation is on the basis of morphology —the cocci in one group, the bacilli in three subgroups: one for those rod-shaped organisms showing branching and curving forms, one for the spore bearers and one for the simple rods. The spirilla are grouped by themselves.

An important method of differentiation is the reaction to Gram's stain. It should be remembered that organisms carried along on artificial media often lose their Gram staining characteristics; hence it is desirable to determine this staining reaction in cultures freshly isolated. Be sure that the stains, especially the aniline gentian violet and the iodine solution, have not deteriorated. There is no more important stain than this, and none which requires greater experience. The chief causes of conflicting results are 1. working with old cultures and 2. not having satisfactory staining solutions.

Motility, as stated above, is at times difficult to determine. For this purpose young eighteen-hour-old bouillion cultures are preferable, and the preparation should be made by applying a vaseline ring to the slide, then putting a drop of the bouillion culture in the center of the ring (or a drop of water inoculated from an agar slant growth), then putting on a cover-glass. By this method current movement is done away with and the preparation keeps for hours. This is a convenient method for agglutination tests.

Liquefaction of gelatin is a very important means of differentiating. When a room-temperature incubator is not at hand (20° to 22° C.), it is better to put the inoculated gelatin tube in the body-temperature incubator, and from day to day test the power of solidifying with ice-water. If the organism digests the gelatin (a liquefier), the medium will remain fluid when placed in ice-water; if the organism is a nonliquefier, the medium in the tube becomes solid. Of course we lose the information to be obtained from the shape of the area of liquefaction.

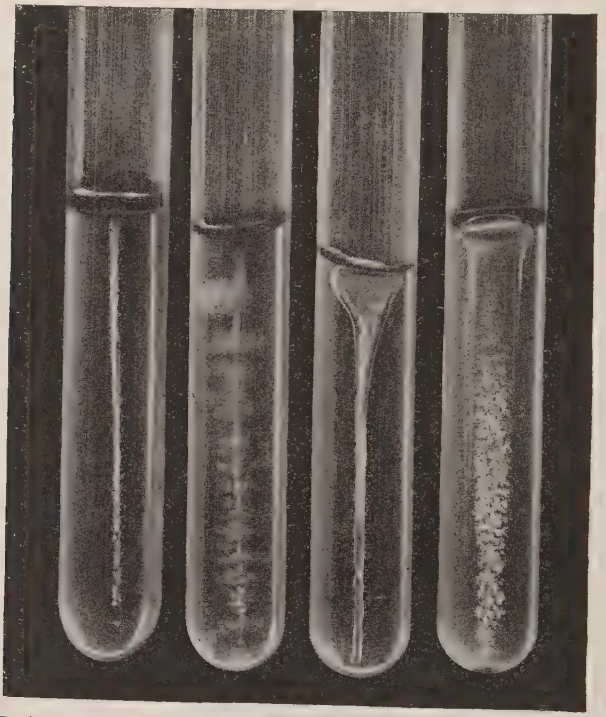

FIG. 10.—Series of stab cultures in gelatin, showing modes of growth of different species of bacteria. (*Abbott.*)

For routine work the only sugar media used are the glucose and the lactose bouillon. These are of the utmost importance in differentiating organisms of the typhoid and colon group. Following Ford, these intestinal bacteria have primarily been separated by their action on litmus milk—whether turning it pink or only slightly changing or not changing at all the original color.

Examine the colonies on Petri plate at first with the unaided eye, then with a hand magnifying glass or low-power objective, using re-

flected and transmitted light alternately. Having determined the presence of two or more different kinds of colonies, make a ring with wax pencil around one or more of each kind of colony, numbering them. The slides or culture tubes used in determining the species of organism present in the plate should bear the same number as that of the colony from which the material was taken. A convenient procedure is to put a loopful of water on a clean cover-glass and to emulsify material from a colony in it. Then invert over a concave slide without vaselining the circumference of the concavity. After examining for motility, smear out and dry the bacterial preparation. Then fix in the flame and stain with aniline gentian violet for two to five minutes. Wash and mount the preparation in water. Afterward pass through the usual Gram technic.

After this inoculate the various culture media from similar colonies. One may inoculate a tube of bouillon from a single colony and later on inoculate the other culture tubes.

In testing for gas production it is better to use the Durham fermentation tube as small amounts of gas may not be easily detected with deep stab cultures into glucose or lactose agar.

If a Durham or Smith tube be not at hand the production of gas may be determined by observing bubble formation on the surface of the sugar bouillon culture. As none of the pathogenic cocci produce gas, fermentation tubes are unnecessary where cocci are to be studied. The litmus milk tube gives data as to acid production.

An important point is to wait at least forty-eight hours (in the case of M. melitensis, four to seven days) before reporting on the cultural findings on the agar or blood-serum slant or plate upon which the material is smeared (pus, exudate, blood, etc.).

Should an organism be encountered in original investigations these requirements as to etiological relationship should be carried out (Koch's postulates). 1. The organism should be constantly present in that particular pathological condition. 2. Such bacteria should be isolated in pure culture from the pathological material. 3. Such pure cultures when inoculated into suitable animals should reproduce the pathological conditions and should be capable of a second isolation in pure culture from such an experimental animal. For various reasons, such as unsuitable animals or artificial media, these requirements are impossible of execution with several organisms which are generally recognized as the causes of certain diseases.

The experimental animals most frequently employed in the diagno-

sis of bacterial diseases are the guinea pig, the rabbit, the white rat and the white mouse. In the following diseases the most suitable animals for inoculation are:

1. Tetanus—mice or guinea-pigs subcutaneously. The spasms begin in the limbs nearest the site of inoculation.
2. Pneumococci and streptococci—mice intraperitoneally or rabbits intravenously.
3. Staphylococci—rabbits.
4. Diphtheria, tuberculosis, anthrax and malignant œdema—the guinea-pig subcutaneously.
5. Glanders and cholera—the guinea pig, intraperitoneally.
6. Plague—guinea-pigs, cutaneously or subcutaneously.

In the cutaneous method of infection the material, as from a plague bubo, or the sputum from pneumonic plague, is thoroughly rubbed with a glass rod upon the shaven surface of the guinea-pig.

In the subcutaneous method one can use a hypodermic needle (the all glass syringe with platino-iridium needle is the best) or an opening can be cut with the scissors, a pocket then opened up with the forceps and a piece of tissue inserted to the bottom of the pocket with the forceps.

The large ear vein of the rabbit is used for intravenous inoculation. This can be made to stand out with either hot water or xylol.

In intraperitoneal injections the animal is best held head down so that the intestines gravitate downward. The shaven skin is pinched up and the needle inserted in the median line.

CHAPTER V.

STUDY AND IDENTIFICATION OF BACTERIA—COCCI. KEY AND NOTES.

Streptococcus Forms.—Cells divide to form chains.

I. Gelatin not liquefied.

1. Hæmolytic zone on blood agar.

a. Very slight acidity in lactose litmus bouillon. S. pyogenes. Tends to produce arthritis in experimental animals. Often a granular sediment in bouillon.

b. Marked acidity but no gas production in lactose litmus bouillon. S. acidi lactici. Non pathogenic. Forms diffuse cloudiness ín bouillon.

2. Greenish appearance about colonies on blood agar.

a. No tendency to capsule formation. S. viridans. Produces endocarditis in experimental animals.

b. Distinct capsule formation in pathological material or on favorable media. S. lanceolatus (Pneumococcus). Gram positive, lance-shaped cocci with bases apposed within a capsule.

c. Very marked capsule development on all media. S. mucosus. A. streptococcus with extraordinary capsule development, up to 10μ in width, S. mesenterioides, is not pathogenic.

II. Gelatin liquefied.

Streptococcus coli gracilis. (Cocci quite small—0.2 to 0.4μ. In fæces.)

A tube-like liquefaction, chains rather long; only slight growth on agar. Constant inhabitant of stools of meat diet.

Sarcina Forms.—Cells divide in three dimensions of space. (Packets).

A. No pigment production on agar.

a. Sarcina alba. (Colonies finely granular.)

b. Sarcina pulmonum.

B. Yellowish pigment.

a. Sarcina lutea. (Colonies coarsely granular.)

b. Sarcina flava. (Colonies finely granular.)

C. Rose-red pigment.

a. Sarcina rosea.

Micrococcus Forms.—Cells divide irregularly in various directions.

I. Gram-positive cocci.

A. Cocci—round.

1. Divide in two planes at right angles. Tetrad formation. Merismopedia.

a. M. tetragenus. Moist white viscid colones. No liquefaction of gelatin. Capsule.

2. Divide irregularly. Bunch of grapes arrangement. (Staphylococci.)
   a. Gelatin not liquefied. M. cereus albus.
   b. Gelatin liquefied. { M. (Staphylococcus) pyogenes albus. / M. (Staphylococcus) pyogenes aureus.
   c. Gelatin very slightly liquefied.
      S. epidermidis albus. (Stitch coccus.)

B. Cocci—biscuit-shape.
   Diplococcus crassus. (May be mistaken for meningococcus.)

On ordinary agar we have a scanty growth resembling the streptococcus. Colonies on ascites agar are smaller than those of meningococcus. It produces acid in glucose, maltose and lactose.

II. Gram-negative cocci.
   A. Grow only at about incubator temperature.
      1. Grow only on blood or serum media. Gonococcus.
      2. Grow on blood serum media, or glycerine agar.
         a. Diplococcus intracellularis meningitidis. (Produces acid in glucose and maltose but not in lactose.)
      3. Grows on ordinary media. Micrococcus melitensis.
   B. Will grow at room temperature as well as at 37° C.
      a. Micrococcus catarrhalis. Does not produce acid in glucose or maltose.
      b. M. pharyngis siccus. Colonies dry and tough and adhere to medium.

NOTE.—Other biscuit-shaped Gram negative organisms resembling the meningococcus are (a) Diplococcus flavus. The colonies show yellow pigment and we have three varieties according to the depth of the yellow color. (b) M. pharyngis siccus and (c) M. cinereus chiefly have coarse dry colonies on ascitic agar.

STREPTOCOCCUS FORMS.

Those cocci tending to arrange themselves in chains are usually described as streptococci. (Ogston, 1881; Rosenbach, 1884.)

When we consider that certain bacilli at times assume an arrangement which we term strepto-bacilli, yet have no relationship, it would suggest that the matter of chain morphology is simply a characteristic common to many entirely different cocci.

Again old laboratory cultures of streptococci may show alternations of cocci and rods giving the appearance of the dots and dashes of the Morse code. Furthermore unsuitable media may bring about various involution types in an organism primarily streptococcal.

It is often difficult to distinguish streptobacilli from streptococci morphologically and the same is true of diplococci and diplobacilli. These bacillary pairs and chains however often show bipolar staining and are almost invariably Gram negative.

While streptococci tend to assume chain formation in pus and tissues they often appear as diplococci in blood.

The essential point to bear in mind is that the finding of a streptococcus does not necessarily explain an infection, because normally streptococci are among the organisms most frequently and abundantly found in plates made from normal buccal and nasal secretions. It is well to be very conservative when reporting streptococci as the etiological factor from lesions of the throat or nose.

Probably the most practical point in the differentiation of streptococci, next to that of pathogenicity, is the occurrence of long or short chains, the virulent ones tending to appear in chains of from ten to twenty cocci, while the normal inhabitants of the nose, mouth and fæces generally tend to be in shorter chains.

FIG. 11—Streptococcus pyogenes. (*Kolle and Wassermann.*)

As regards virulence, this is exceedingly variable—it is soon lost, but may be restored either by inoculating streptococci along with various other organisms or by passage through successive rabbits. The rabbit is the most susceptible animal and should be inoculated in one of the prominent ear veins. If the needle of the syringe is not inserted in the vein it will be difficult to force in the material and a swelling will immediately show itself.

Besides the morphological and pathogenic variations, Schottmuller has noted differences where these organisms are grown on 1 part of blood and 3 to 6 parts of agar. On this medium Strep. erysipelatis has a hemolytic action, the laking of the red cells bringing about a more or less clear ring surrounding the colony. The short-chain streptococci do not have a hemolytic halo. The pneumococcus has a

greenish zone. Streptococci which are profoundly toxic and which have been isolated from milk-borne epidemic sore throats differ from the ordinary S. pyogenes in being encapsulated, not tending to form chains and producing only slight hæmolysis on blood agar.

Some of the English authorities have introduced biochemical methods of differentiating: the Strep. pyogenes coagulating milk, reducing neutral red, and producing acid in lactose, saccharose, and mannite media.

S. pyogenes does not produce acid in inulin media while the pneumococcus does.

A freshly prepared solution of sodium taurocholate, 5%, added to an equal amount of a twenty-four-hour bouillon culture of S. pyogenes does not disintegrate the cocci or, at any rate, not within a few minutes. The reverse is true of the pneumococcus.

When we consider the biochemical variations which a single organism, as the colon bacillus, may exhibit, the value of such methods of differentiating may well be questioned. The question of the symbiotic relationship, which, when established between two or more bacteria, may cause harmless organisms to take on virulence, would appear to be a more important consideration.

Almost without exception, human streptococci are Gram positive. Their colonies are quite small, but distinct and discrete. In appearance the colonies of streptococci and pneumococci are practically identical. In a blood-serum throat culture pneumococcus and streptococcus colonies are the smallest, diphtheria ones are quite small and discrete, but slightly flatter. (Always examine the water of condensation for streptococci.) The sarcina and staphylococcus colonies are much larger.

Streptococcic colonies on blood agar are much more moist and luxuriant than on ordinary agar. A very important point, in judging whether a streptococcus or other organism is pathogenic in a given infection, is to examine smears from the pus or other material in a Gram-stained specimen for information as to abundance and, in particular, phagocytosis of any organism, before plating out.

Streptococci are commonly the cause of diffuse phlegmonous inflammations, while the staphylococci cause circumscribed lesions. Streptococci cause necrosis and do not characteristically produce pus. The importance of the streptococcus as a secondary infection in diphtheria, tuberculosis, small-pox, and even in typhoid fever must always be kept in mind. It is this infection which does not respond to diphtheria antitoxin, and not the diphtheria one.

When freshly isolated from human lesions streptococci often show only a slight virulence for animals. Hence massive doses are indicated and intravenous or intraperitoneal injections. The guinea-pig is not very susceptible to streptococci; the rabbit and white mouse being the animals of choice.

In nondiphtheritic anginas, puerperal fever, ulcerative endocarditis and coccal enteritis it is the streptococcus which is usually the cause. It has been claimed that acute articular rheumatism is due to a short-chain streptococcus (M. rheumaticus), which is best isolated from material from an acute joint infection, but may also be isolated occasionally from the blood. It produces much acid and clots milk in two days. The growth is described as being more luxuriant than that of S. pyogenes. It is about 0.5μ in diameter.

The majority of investigators have reported streptococci from acute joint inflammations and bacilli from chronic infectious joint affections. Goadby has considered a streptobacillus, somewhat resembling Ducrey's bacillus of chancroid, which exhibits marked pleomorphism and Gram variations, and grows best on egg albumin agar of plus 3 reaction as the cause of arthritis deformans and alveolar osteitis. Inoculation of cultures of this organism into or around the knee-joints of rabbits has produced lesions similar to those of rheumatoid arthritis.

Sarcina Forms.

These are best observed in hanging-drop preparations, when they can be seen as little cubes, like a parcel tied with a string, and by noting them when turning over, it will be seen that they are different from the tetrads which only divide in two directions of space. At times the packet formation is not perfect and it will be difficult to distinguish such as sarcinæ. All sarcinæ stain by Gram. If the staining of sarcinæ be too deep it may obscure the lines of cleavage. Sarcinæ are nonmotile.

Various sarcinæ have been isolated from the stomach, especially when there is stagnation of stomach contents. Sarcinæ have also been found in the intestines. In plates the S. lutea is frequently a contaminating organism, being rather constantly present in the air. The demonstration of sarcina morphology should always be made from liquid media, as bouillon. Urine makes an excellent medium.

Micrococcus Forms.

This grouping includes all cocci which do not show chain or packet formation. It will be found convenient to divide them into two classes

according to their staining by Gram. The M. tetragenus, S. pyogenes aureus and the pneumococcus stain by Gram, while the gonococcus, the meningococcus, the M. catarrhalis and the M. melitensis are Gram negative.

M. tetragenus.—This organism is frequently found associated with other organisms in sputum, especially with tubercle and influenza bacilli. The colonies are white, slightly smaller than staphylococci and are quite viscid.

FIG. 12.—Gelatine culture Staphylococcus aureus one week old. (*Williams.*)

It was formerly considered unimportant in disease, but the idea now prevails that it is responsible for many abscesses about the mouth, especially in connection with the teeth. Injected subcutaneously into mice, it produces a septicæmia and death in three or four days. The blood shows great numbers of encapsulated tetrads. It has been reported twice as a cause of septicæmia in man.

Staphylococci.—To cocci dividing irregularly and usually forming masses which are likened to clusters of grapes the term staphylococcus is applied. While there have been experiments which show that by selecting pale portions of a yellow colony, eventually a white colony could be produced, yet, as a practical consideration, it is convenient to consider at least two types of staphylococci: the Staphylococcus pyogenes aureus and the Staphylococcus pyogenes albus. In culturing from the pus of an abscess or furuncle we generally obtain a golden coccus, while in material from the nose or mouth, the staphylococcus colonies are almost invariably white. As regards the common skin coccus, this will be found to produce a white colony. A coccus which very slowly liquefies gelatin and has been supposed to cause stitch abscesses is the S. epidermidis albus.

While it is customary to look for a golden colony in the case of organisms showing virulence, yet at times a cream-white colony may develop from cocci of great virulence.

The S. pyogenes citreus is considered as of very feeble pathogenic power. Certain cocci whose colonies have presented a waxy appearence have been designated as S. cereus albus and S. cereus flavus, respectively. They are of very little practi-

cal importance. The Staphylococcus pyogenes aureus grows readily at room temperature, but better at 37° C. It coagulates milk and renders bouillon uniformly turbid. It grows on all media, as blood-serum, agar, potato, etc. It has been proposed to distinguish it from skin staphylococci by its power of producing acid in mannite. Ordinarily the individual cocci are about 1μ in diameter, but they vary greatly in size according to the age of the culture and other conditions. The aureus, as it is frequently called, is not only often found in circumscribed processes, but it is a frequent cause of septicæmia, osteomyelitis, endocarditis, etc.

In infection of bone tissue the staphylococcus is by far the most frequent cause. It is well to remember that insignificant staphylococcal infection may lead to septicæmia. In the tropics, where resistance is often lowered and staphylococcal skin infections common, continued fevers are often septicæmias. It is the organism most frequently concerned in terminal infections. The lowered resistance of the patient permits of their passage through barriers ordinarily resistant. Not only should this be kept in mind when such organisms are isolated at an autopsy, but as well the fact that their entrance may have been agonal or subsequent to death.

The Pneumococcus of Fraenkel.—(Weichselbaum differentiated organisms causing pneumonia in 1886.) This is by far the most common cause of pneumonia, whether it be of the croupous, catarrhal, or septic type. It is also frequently found in meningitis, empyema, endocarditis and otitis media. It should not be confused with the pneumobacillus of Friedländer, which, although possessing a capsule like the pneumococcus, differs from it by being Gram negative, being a bacillus and having large viscid colonies. The pneumococcus is the cause of more than 80% of the cases of pneumonia. It does not grow below 20° C. and is best cultivated on blood-serum, or blood-streaked agar. On plain agar it grows as a very small dew-drop-like colony, which is slightly grayish by reflected light. It produces considerable acid, thus acidifying and usually coagulating litmus milk. It produces acid in inulin media which the streptococcus fails to do. The colony is smaller and more transparent than a streptococcus colony. In sputum or other pathological material it is best recognized by the presence of a capsule inclosed in which are two lance-shaped cocci with their bases apposed. In artificial culture we rarely get the capsule. It also sometimes grows in short chains like a streptococcus. The best medium for differentiating is the serum of a young rabbit; in this it grows as a diplococcus, while streptococci show chains. The best method of isolating it in pure culture is to inject the sputum into the marginal ear vein of a rabbit or subcutaneously into a mouse. Death results from septicæmia in about two days and the blood teems with pneumococci. Usually the pneumococcus quickly loses its virulence, and

also dies out in a few days unless transferred to fresh media. The best medium for its preservation is rabbit's blood agar; this also maintains the virulence. On this medium the colonies are larger than on agar and they present a greenish appearance.

The pneumococcus growth emulsifies very readily and evenly so that suspensions for vaccines are easily made.

It is a well-known fact that the pneumococcus is a frequent inhabitant of the nasal, pharyngeal, and buccal cavities. The explanation of infection is either on the ground of lowered resistance of the patient or enhanced virulence of the organism. Oscar Richardson has reported an organism in cases of lobar pneumonia, cerebrospinal meningitis, mastoid disease, etc., bearing resemblance to both pneumococci and streptococci—the Streptococcus capsulatus. It differs from the pneumococcus

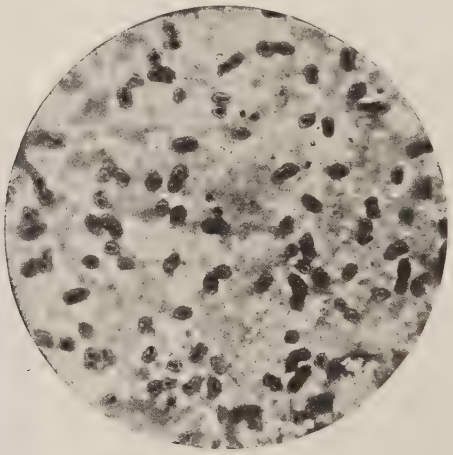

FIG. 13.—Pneumococcus, showing capsule, from pleuritic fluid of infected rabbit, stained by second method of Hiss. (*Williams.*)

in that the colonies on blood-serum are viscid and like irregular flecks of mucus. The characteristic culture is a glucose agar stab. (Reaction must not exceed +0.5.) From the line of puncture there are flail-like projections extending outward from one-fifth to one-fourth of an inch. The capsule persists on ordinary culture media. This organism resembles the Streptococcus of Bonome of the French.

In a study of blood and sputum cultures from thirty-two cases of lobar pneumonia Hastings and Boehm found blood and sputum positive bacteriologically in eleven cases. In nine of these cases the pneumococcus was isolated and in two a hæmolysing streptococcus. In the other twenty-one cases the sputum cultures were bacteriologically positive in eighteen of the cases and negative in three. In nine cases the pneumococcus was isolated, in two cases B. coli, in one case M. catarrhalis, in one case a staphylococcus, in two cases staphylococci and streptococci, in one case B. influenzæ. The percentage of positive blood

cultures was 30.3. Cole obtained 30% of positive blood cultures. The blood was taken into flasks of bouillon in dilution of 1–50.

Diplococcus crassus.—This is a Gram positive, kidney-shaped diplococcus, which might be confused with the M. catarrhalis or the meningococcus by ordinary staining methods. It is larger than the meningococcus.

In throat cultures I have isolated on several occasions a Gram positive diplococcus which is at times biscuit-shaped, at times irregularly spherical. It possesses two or three metachromatic granules, so that in a Neisser stain for diphtheria the appearance of these granules may be confusing.

Using Ponder's toluidin blue stain I have observed granule staining in organisms of round or oval morphology which were suggestive of the ascospore staining of yeasts.

Gram Negative Cocci.—It is important to bear in mind that there are many cocci of varying shapes, which in cultures or in smears from the throat, nose or fæces are Gram negative. These are not well classified or described. To distinguish the three important kidney-shaped diplococci, it can be most easily accomplished by cultural methods, using hydrocele agar (ascites or blood agar will answer), ordinary blood-serum and plain agar. The gonococcus will only grow on the hydrocele agar; the meningococcus will grow on this, but likewise grows on ordinary blood-serum. The M. catarrhalis will grow on plain agar as well as on other media.

Other Gram negative organisms of confusing morphology are M. pharyngis siccus, the colonies of which show great crinkly dryness, and M. pharyngis flavus.

Gonococcus (Neisser, 1879).—This organism is characteristically a diplococcus, the separate cocci being plano-convex with their plane surfaces apposed. (Biscuit shape, coffee-bean shape.) They are generally found grouped in masses of several pairs, most strikingly in pus cells or epithelial cells, but also found extracellularly. Except in the height of the disease, there is a great tendency for the organisms to show involution forms, so that instead of biscuit-shaped diplococci we have round, irregular and uneven cocci. It is therefore advisable in searching smears from chronic gonorrhœa to continue the search of Gram-stained specimens until some fairly typical diplococci are found. There is nothing requiring greater discrimination than a diagnosis from such a smear. At the commencement of a gonorrhœa the epithelial cells are abundant and gonococci are found adhering to them or lying free.

Later on, at the acme of the discharge (the creamy, abundant discharge), it is in the pus cells we find them and they may be so abundant that 10 to 20% of the pus cells may contain them. In the subacute stage the epithelial cells, which practically disappear when the discharge is so abundant, begin to reappear, and in the chronic stage the epithelial cells are the chief ones, and are the ones, on which we find an occasional gonococcus, often distorted in shape.

The best method of diagnosis in cases of chronic gonorrhœa is to have the patient drink beer and eat the stimulating food previously interdicted, to take active exercise and to have a sound passed. To obtain material for examination the glans penis should be washed and the patient who has presented himself with a full bladder should pass a portion of the contained urine. Next the prostate and seminal

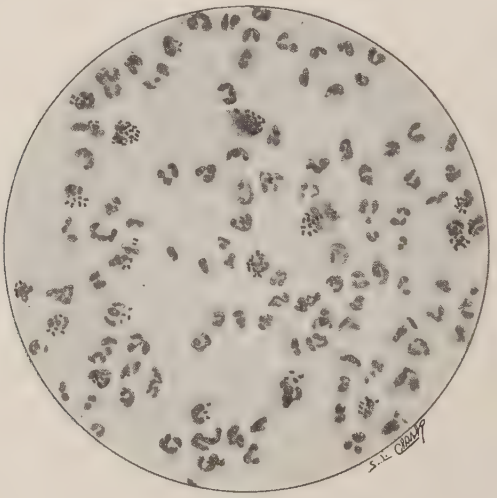

FIG. 14.—Gonococcus. Film from urethral pus. (*Coplin.*)

vesicles should be massaged with the patient standing but bent over and the penis pendant. The drops of discharge from the massage should be received in a small Petri dish and finally the remaining urine should be passed into a sterile bottle. Smears and cultures should be made from the sediment of the two urinary specimens and from the secretions of the massaged prostate and vesicles.

The smears made from the resulting discharge or centrifuged urine will probably contain gonoccocci if they are present in the urethra. In the female the favorite sites are the urethra and the cervix uteri. In municipal examinations it is customary to make two smears: one from the urethral meatus and a second from the cervix. The vagina is not a suitable soil for their development. In female children it is most often found in the discharge of the vulvovaginitis.

In addition to the genital organs, the gonococcus may at times invade and be isolated from the eye (gonorrhœal ophthalmia), the joints, rarely as a cause of endocarditis and possibly as the factor in septicæmia. Grown upon hydrocele or ascites agar, or blood-streaked agar, or upon blood agar from man or the rabbit, the colonies appear as irregular, minute, dew-drop spots. By the second or third day the involution forms are abundant, and within four to seven days the culture will probably be found to be dead. Unless frequent transfers are made, it will be best kept alive on blood agar. The organism grows best at 37° C., and will not grow below 25° C. It will not grow on plain or glycerine agar or ordinary blood-serum unless the transfer of considerable pus in inoculating the slants gives it a suitable culture medium. In material from joints, it is in the fibrin flakes that the gonococci are most apt to be found, if found at all.

By heating the blood-streaked agar tubes to 56° C. for twenty minutes (inactivation-destroying complement and hence bactericidal power of blood on slant) greater success in primary cultures will be obtained.

In culturing gonococci the transfer of material to culture media should be made with the least delay possible.

The most satisfactory medium is Thalman's medium upon the slanting surface of which we have deposited two or three drops of human serum. Blood may be taken from a vein or the Wright U tube may be used and after centrifuging the sterile serum is taken off with a capillary bulb pipette and deposited on and smeared out on the slant.

Diplococcus intracellularis meningitidis (Weichselbaum, 1887).—This is the organism of epidemic cerebrospinal meningitis, and is frequently termed the meningococcus. The diplococcus is Gram negative and biscuit-shaped and is, like the gonococcus, chiefly contained in pus cells. It is also found free in the cerebrospinal fluid withdrawn from cerebrospinal fever cases. There is a greater tendency to variation in size and shape than is the case with the gonococcus, which latter, in fresh material, shows a striking uniformity morphologically. The meningococcus is at times not abundant—early in the case, however, the picture may be similar to that of gonorrhœa.

On blood-serum the colonies appear after twenty-four to forty-eight hours as discrete, very slightly hazy colonies, about one-tenth of an inch in diameter. On serum agar, as ascites or hydrocele agar, they grow best. Unless considerable cerebrospinal fluid is transferred with the inoculating loop, they do not grow on plain agar. They will grow at times on glycerine agar. The organism is very sensitive to light, cold and drying. It ferments dextrose and only grows at blood temperature, thus distinguishing it from the M. catarrhalis. It is scarcely pathogenic for laboratory animals, with the exception of the mouse and guinea-pig, when intraperitoneal injections but not subcutaneous ones give results. Intradural injections give results. The cultures die out very rapidly, so that it is necessary to

make transfers every one or two days. The meningococcus has been isolated from the nasal secretions of patients. The possibility of these organisms being the M. catarrhalis must be considered.

Flexner has shown that in monkeys, which are susceptible to the disease, injections of cultures of M. intracellularis into the spinal canal is followed by migration of the cocci to the nasal cavity both free and in phagocytic leukocytes.

The meningococcus has a very slight resistance to sun or drying so that its aërial transmission seems doubtful. It is supposed to effect an entrance by the nares, thence reaching the cerebral meninges. Infec-

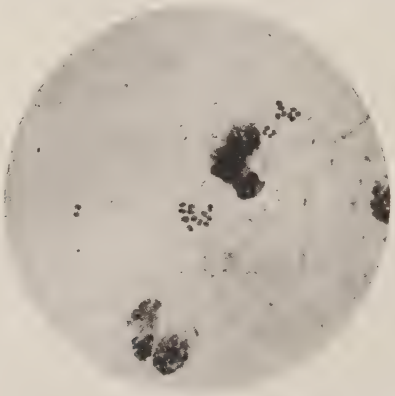

FIG. 15.—Diplococcus intracellularis meningitidis and pus cells. (×1000.) (*Williams.*)

tion is probably by direct contagion. Several cases have been reported where with a high leukocytosis the cocci have been found in the polymorphonuclears of blood smears and in cultures from the blood. (In about 25% of blood cultures where from 5 to 10 c.c. are employed.)

By the use of initial injections into horses of killed cultures followed by alternate injections into horses of living diplococci, then seven days later of an autolysate made from different strains; seven days later again injecting living diplococci; thus alternating material every week, an antiserum of value has been obtained by Flexner. The immunization requires about one year. In using, withdraw about 20 c.c. of patient's cerebrospinal fluid with a syringe, and then inject, through the same needle, an equal quantity of the serum. The injection is repeated every day for three or four days.

For diagnosis, make smears and cultures from cerebrospinal fluid. The sediment from the centrifuged material gives better results. In tuberculosis the lymphocytes preponderate; in cerebrospinal meningitis the polymorphonuclears.

It has been stated that a point of difference between the phagocytosis with the gonoccoci and the meningococci is that the meningococci invade and at times destroy the nucleus of the polymorphonuclear, which is not true of gonococci. The appearance of large phagocytic endothelial cells, often containing polymorphonuclears, in the centrifuged cerebrospinal fluid is a favorable prognostic sign. At times there does not appear to be any relation between the number of phagocytic polymorphonuclears and the severity of the case.

Vincent has recommended a precipitin test for epidemic cerebrospinal meningitis which has the advantages of being simple and more immediate than cultures and of particular value in those cases when meningococci cannot be found in the smears or in cultures from the cerebrospinal fluid. It is performed by adding one or two drops of antimeningococcic serum to a tube of fresh cerebrospinal fluid which has been cleared by centrifugalization for 10 to 15 minutes. After adding the serum the tube is placed in the incubator at 52° C. for two to five hours together with a control tube. The formation of a precipitate (turbidity) shows a postive test.

Micrococcus catarrhalis (Seifert, 1890).—This organism has been specially studied by Lord. It resembles the meningococcus strikingly and can only be differentiated by cultural procedures. It grows on plain agar and at room temperature, and does not produce acid in glucose media. It not only occurs in the nasal secretions of healthy people, but appears to be responsible for certain coryzas and bronchial affections, resembling influenza. It also is responsible for certain epidemics of conjunctivitis.

The original cultures may show only slight growth whereas the subcultures prove luxuriant.

The colonies are larger, more opaque, and have a more irregular wavy border than the round colonies of the meningococcus.

Micrococcus melitensis (Bruce, 1887).—This is the organism of Malta or Mediterranean fever, sometimes called undulant fever, on account of successive waves of pyrexia running over several months. The disease has a very slight mortality (2%), and the lesions are chiefly of the spleen, which is large and diffluent. The organisms can best be isolated from the spleen.

M. melitensis is only about 0.3μ in diameter. The characteristics are its very small size and the dew-drop minute colonies on agar, which at incubator temperature only show themselves about the third to the sixth day. It is nonmotile and Gram negative. In bouillon there is a slight turbidity.

Many laboratory infections have been recorded.

The organism occurs in peripheral circulation, it having been cultivated from blood very successfully by Eyre. He takes blood at the height of the fever, and in the afternoon. Formerly it was customary to isolate by splenic puncture.

Infection is chiefly by means of the milk of infected goats. The organisms are

excreted in the urine of patients, and a diagnostic point is to make plates from the urine. Such urine applied to abraded surfaces causes infection.

The serum of patients shows agglutinating power as early as the fifth day of the disease, and this may persist for years after recovery. Nicolle has advised using serum heated to 56° C. for 30 minutes for the agglutination test, nonspecific agglutinins being thereby destroyed. Carriers may be of importance in Malta fever and are best detected by agglutination tests.

A high mononuclear increase may be found in this disease.

CHAPTER VI.

STUDY AND IDENTIFICATION OF BACTERIA. SPORE-BEARING BACILLI. KEY AND NOTES.

A. **Grow aerobically.**

1. Stab culture in gelatin has branches growing out at right angles to line of stab.
   a. Has no membrane on bouillon or liquefied gelatin. Projecting branches from line of stab only at upper part of line of growth. Absolutely nonmotile, Ends sharply cut across or concave. ANTHRAX GROUP.
   b. Has thick whitish membrane on bouillon and surface of liquified gelatin. Projecting branches all along the line of stab. Sluggishly motile. MYCOIDES GROUP. (B. mycoides. B. ramosus.)
2. Stab cultures in gelatin do not show projecting branches.
   a. Potato cultures do not become wrinkled. At first slightly moist, later dry and mealy. SUBTILIS GROUP. (Hay bacillus group.) Actively motile with more or less square ends and a central spore which is of the same diameter or only slightly larger than the bacillus. The yellow subtilis is at times found in water. The colonies on potato are of a cheese-yellow color. The bacilli are very large and show a sluggish, worm-like motion.
   B. megatherium often shows a granular or beaded appearance in a Gram preparation. The narrow spores are never central, usually between center and end, and rather elongated. It most nearly resembles the sporulating bacillus of malignant œdema but if the spore is quite terminal and bulging may resemble B. tetani.
   Cultures of B. megatherium are somewhat similar to B. coli colonies.
   b. Potato cultures at first even growth but after a few days become wrinkled. VULGATUS GROUP. (Potato bacillus.)
   B. vulgatus shows marked wrinkling, like intestinal coils. B. mesentericus show slight wrinkling and a network-like appearance.
   Two water bacilli belonging to this group are the B. mesentericus fuscus (brown growth) and B. mesentericus ruber (red growth).

NOTE.—The following cultural characteristics are common to all the above spore bearers.

1. Liquefaction of gelatin.
2. Milk slowly and incompletely coagulated with very little change in reaction. Later the coagulum is digested.
3. No gas in either glucose or lactose.
4. No indol.
5. All are Gram positive.
6. All digest blood-serum.

B. **Grow only anaerobically.**
1. Rods very little swollen by centrally situated spores.
   a. Motile. B. œdematis maligni. (Gram negative.)
   b. Non motile. B. aerogenes capsulatus. (Capsule.)
2. Spores tend to be situated between center and end.
   a. No liquefaction of gelatin. B. butyricus.
   b. Gelatin liquefied slowly.
      B. botulinus. Milk not coagulated.
      B. anthracis symptomatici.
      B. enteritidis sporogenes. Milk coagulated with abundant gas.
   c. Gelatin liquefied rapidly. B. cadaveris sporogenes. Very motile.
3. Spores situated at end of rod. Drum-stick sporulation. TETANUS GROUP.

The following table taken from Lehmann and Neumann, based on pathogenic effects, is of great practical value. After inoculation of some animal subcutaneously with the suspected material we have:

A. No particular symptoms at site of inoculation.
   Absorption of the soluble toxin causing:
   (1) General symptoms of tetanus. B. tetani.
   (2) Botulism poisoning symptoms. Pupillary symptoms. Paralysis of tongue and pharynx. Cardiac and respiratory failure.

B. Local symptoms marked at site of inoculation. Hemorrhagic emphysematous œdema.
   (1) Motile.
      (a) Gram negative. B. œdematis maligni.
      (b) Gram positive. B. anthracis symptomatici.
   (2) Nonmotile.
      B. aerogenes capsulatus or B. phlegmonis emphysematosæ.

Spore-bearing Aerobes.

Bacillus anthracis (Pollender discovered 1849. Davaine recognized nature 1863. Koch proved 1876).—Of the aerobic spore-bearing bacilli this is the only one of particular medical importance.

Anthrax is an important disease in domestic animals, especially sheep and cattle. The characteristic postmortem change in animals is the greatly enlarged, friable, mushy spleen. Man is much less susceptible than these animals, but is more so than the goat, horse, or pig. The Algerian sheep has a high degree of immunity, as has the white rat. The brown rat is quite susceptible. The disease in man chiefly occurs among those working with hides, wool, or meat of infected cattle. The two chief types in man are: 1. Malignant pustule and 2. Woolsorter's disease. An intestinal type is also recognized. Malignant pustule

results from the inoculation of an abrasion or cut; thus it frequently shows on the arms and the backs of those unloading hides. It first appears as a pimple, the center of which becomes vesicular, then necrotic.

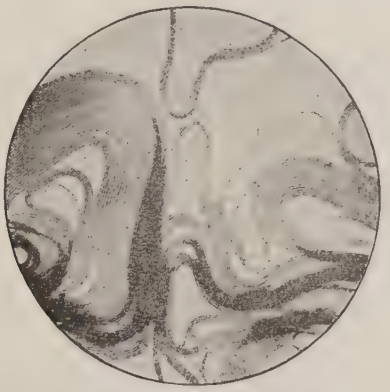

FIG. 16.—Anthrax bacilli. Cover-glass has been pressed on a colony and then fixed and stained. (*Kolle and Wassermann.*)

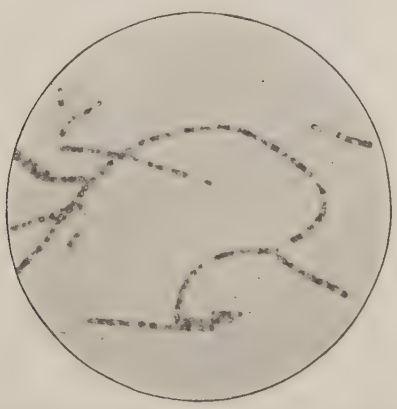

FIG. 17.—Anthrax bacilli growing in a chain and exhibiting spores. (*Kolle and Wasserman.*)

A ring of vesicles surrounds this central eschar and a zone of congestion, the vesicles. The lymphatics soon become inflamed as well as neighboring glands. If the pustule is not excised and death occurs, there is not much enlargement of the spleen and the bacteria are not abundant in the kidneys, etc., as with animals. Man seems to die from a toxæmia rather than a septicæmia.

In woolsorter's disease there is great swelling and œdema of the bronchial and mediastinal glands. The lungs show œdema, which about the bronchi is hemorrhagic.

The bacillus is 5 to 8μ by 1 to 1 1/2μ. It has square cut or concave ends and is often found in chains. It is Gram positive. Colonies, by interlacing waves of strings of bacteria, show Medusa head appearance. For cultural characteristics see key. Spores develop best at a temperature of 30° C. They stain with difficulty.

Stiles thinks that animals are infected by eating the bones of animals which have died of anthrax, cutting buccal mucous membrane, and so becoming infected. Spores do not form in an intact animal body, but they do form after a postmortem or the disintegration of the body by maggots. For this reason it is better not to open up the body of the animal, but to make the diagnosis by cutting off an ear. Dried spores will live for years and will withstand boiling temperature for hours.

In vaccinating animals against anthrax, Pasteur used two vaccines. The first is attenuated fifteen days at 42.5° C. The second, attenuated for only ten days, is given twelve days later.

FIG. 18.—Bacillus anthracis in blood of rabbit. (*Coplin.*)

In taking material from a malignant pustule before excision, be careful not to manipulate it roughly, as bacteria may enter the circulation. Make cover-glass preparations, staining by Gram. Make culture on agar. Blood cultures are usually only positive later in the disease. Inoculate a guinea-pig or a mouse subcutaneously.

The guinea-pig dies in about forty-eight hours and shows an œdematous gelatinous exudate at site of inoculation. The blood is black and swarms with anthrax bacilli. It is the best example of a septicæmia.

An organism with a central spore and morphologically resembling B. anthracis, but motile, has been reported as occurring in the stools of pellagrins. Gelatine stabs show a cup-shaped liquefaction in about

five days. No change in milk. The colonies are slimy and opaque. The organism is said to be agglutinated by the serum of pellagra cases. The name B. MAYDIS has been given to it.

Spore-bearing Anaerobes.

There are three very important pathogens in this group—that of malignant œdema; that of botulism, and the organism of tetanus.

The B. enteritidis sporogenes is of importance in connection with indications of fæcal contamination of water. In connection with B. aerogenes capsulatus, there is some question as to whether the extensive œdema produced by it may not usually be from a terminal or cadaveric infection. At any rate necrotic material seems necessary.

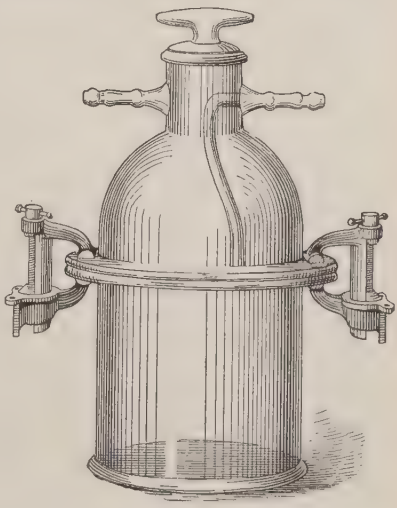

FIG. 19.—Novy jar.

It should be stated that our knowledge of the differential cultural characteristics of anaerobes is unsatisfactory. The exact methods which are in use for aerobes have not been applied to anaerobic organisms.

To Cultivate Anaerobes.—Probably the apparatus giving the most perfect anaerobic conditions is the Novy jar, in which the air has been replaced by hydrogen. The difficulties attending the method are:

1. Unless a special apparatus (Kipp's) is at hand, there may be difficulty in preventing the sulphuric acid from frothing over when poured on the zinc. It should, at first, be added in small quantities at a time—well diluted (1 to 6).
2. Various wash-bottles are required: one containing silver nitrate solution for traces of AsH_3 and one with lead acetate for H_2S and another with pyrogallic acid and caustic soda for any oxygen that may come over.
3. Mixtures of hydrogen and air explode. Consequently, in determining whether all air has been expelled and in its place an atmosphere of hydrogen exists, it is necessary to see if the escaping gas burns with a blue flame. Unless this is collected in a test-tube and examined, we may have an explosion.
4. Except in a large laboratory, where the apparatus is set up and ready for use, too much time would be required.
5. Simpler methods appear to give as good results.

In Tarozzi's method, pieces of fresh sterile organs are added to bouillon. Pieces of kidney, liver, or spleen are best suited. After adding the tissue the media may be heated to 80° C. for a few minutes without interfering with the anaerobic condition producing properties of the fresh tissues. This method is practically the same as that recommended by Smith (see Tetanus). This is also a feature of Noguchi's method of culturing Treponema pallidum.

The Method of Liborius.

In this it is necessary to have a test-tube containing about 4 inches of a 1% glucose agar. Glucose acts as a reducing agent and furnishes energy. It is convenient to add about 1/10 of 1% of sulphindigotate of soda; the loss of the blue color at the site of the colony enabling us to pick them out. The tube of agar should be boiled just before using to expel remaining oxygen from the tube. Now rapidly bring down the temperature to about 42° C., by placing the tube in cold water, and inoculate the material to be examined. A second or third tube may be inoculated from the first, just as in ordinary diluting methods for plate cultures. Having inoculated the tubes, solidify them as quickly as possible, using tap water or ice-water. The anaerobic growth develops in the depths of the medium. Some pour a little sterile vaseline or paraffin or additional agar on the top of the medium in the tube as a seal from the air. Others have recommended the inoculation of some aerobe, as B. prodigiosus, on the surface. This latter method is not advisable. A deep stab culture is often sufficient.

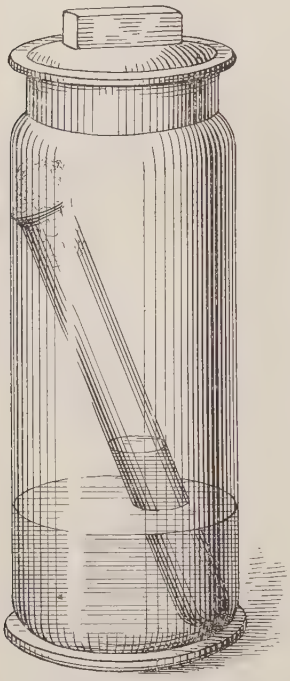

FIG. 20.—Arrangement of tubes for cultivation of anaerobes by Buchner's method. (*Williams.*)

The Method of Buchner.

In this method one gram each of pyrogallic acid and caustic potash or soda for every 100 c.c. of space in the vessel containing the culture is used to absorb the oxygen. It is convenient to drop in the pyrogallic acid; then put in place the inoculated tubes or plates; then quickly pouring in the amount of caustic soda, in a 10% aqueous solution, to immediately close the containing vessel. A large test-tube in which a smaller one containing the inoculated medium is placed, and which may be closed by a rubber stopper, is very convenient. A good rubber-band fruit jar is satisfactory. A desiccator may be used for plates.

An excellent method for anaerobic plates, either in a desiccator with the pyrogallic acid and caustic soda, or less satisfactorily in the open air, is to sterilize the parts of the Petri dish inverted; that is, the smaller part is put bottom downward in the inverted cover (as one would set one tumbler in another). Then, in using, unwrap the Petri dish, lift up the inner part, pour in the inoculated medium into the upturned cover. Then immediately press down the inner dish, spreading out a thin film of the medium between the two bottoms.

J. H. Wright's Method.

Make a deep stab culture in glucose agar or gelatin, preferably boiling the media before inoculating. Then flame the cotton plug and press it down into the tube so that the top lies about three-fourths of an inch below the mouth of the test-tube. Next fill in about one-fourth of an inch with pyrogallic acid; then add 2 or 3 c.c. of a 10% solution of caustic soda, and quickly insert a rubber stopper. This method is one of the most convenient and practical, and is to be strongly recommended.

Method of Vignal.

In this a section of glass tubing (1/4 in.) is drawn out at either end, as in making a bacteriological pipette, with a mouth-piece containing a cotton plug. The liquid agar or gelatin is then inoculated and the medium drawn up into the tube. In a very small flame the capillary narrowings are sealed off, and we have inside the tube very satisfactory anaerobic conditions. To get at the colonies, file a place on the tube and break at this point.

To obtain material for examination and isolation in pure culture from the deep agar stab-tube, it is best to loosen the medium at the sides of the tube with a heated platinum spud or a flattened copper wire. Then shake the mass out into a sterile Petri dish. It is dangerous to break the tubes with a hammer as some do.

A Combination Method.

Recently as shown in the illustration in Fig. 7, I have been combining various methods so that very satisfactory anaerobic conditions are obtained. First, a deep agar stab of freshly sterilized glucose agar is made. The surface of this is then covered with sterile paraffin oil. The proper amount of pyrogallic acid is then deposited in a salt mouth bottle. The rubber stopper with the glass and rubber tubing is then firmly pushed in and connection made with a filter pump.

In five to ten minutes almost all the air will be exhausted when the Hofmann clamp is screwed up tight and the bottle disconnected from the vacuum pump. The glass tubing end is then inserted into a graduate holding 10% caustic soda solution, the Hofmann clamp unscrewed, and the necessary amount of caustic soda having been run in, as noted under Buchner method, we again close the screw clamp and incubate.

B. œdematis maligni (Pasteur, 1877).—This is the vibrion septique of Pasteur. It is found in garden soil and in street sweepings. It is

the cause of an acute cellular necrosis attended with serous sanguinolent exudation and with more or less emphysema. The organism only becomes generalized in the blood about the time of death and postmortem. Therefore, it is not a septicæmia, as is anthrax. The bacillus is an organism about the size of anthrax (7 μ by 0.8), but is narrower and does not have the same square cut or dimpled ends. Furthermore, it is motile, Gram negative and an anaerobe. The guinea-pig is very susceptible, and about the time of death and postmortem there may be seen long flexile motile filaments, 15 to 40 μ long, which move among the blood cells as a serpent in the grass (Pasteur).

In cultures it grows out very slightly from the line of stab, giving a jagged granular line, differing from tetanus. Spores form best at 37° C.—requiring about forty-eight hours. It liquefies gelatin. In examining an exudate from a suspected case, note the presence of spores centrally situated. Inoculate a guinea-pig. Death occurs in about two days. There is intense hemorrhagic emphysematous œdema at the site of inoculation, the œdematous fluid however does not show spores. The bacilli do not appear in the blood until about the time of death and it is an assistance in diagnosis to put the dead body of the guinea-pig in the incubator for a few hours. The subcutaneous tissue contains fluid and gas. There is present the foul odor of an anaerobe. Examine for the long filaments showing flowing motility. Be sure to stain by Gram. (Negative.) For cultures, heat the material (either from a wound or from a guinea-pig) which shows spores to a temperature of 80° C. for from fifteen minutes to one hour. Then inoculate glucose agar stab culture and grow anaerobically. Courmont differentiates anthrax from malignant œdema by injecting into ear-vein of rabbit. The injection of malignant œdema in this way, instead of subcutaneously, tends to immunize.

B. botulinus (Van Ermengem, 1896).—This is the organism which produces botulism, a form of meat poisoning. It is a spore-bearing anaerobe and must not be confused with another organism associated with meat poisoning—the B. enteritidis of Gärtner. The spores are at the end and are not very resistant; a temperature of 80° C. often killing them.

In botulism the meat becomes infected after the animal has been slaughtered; in Gärtner meat poisoning the cow meat was infected at the time of slaughter—it was from a sick animal. Thorough cooking of the meat protects against botulism but not certainly against Gärtner meat poisoning.

There are dysphagia, paralysis of eye-muscles, and cardiac and respiratory symptoms (medulla). The symptoms are due to the elaboration of a soluble toxin of the same nature as that of diphtheria and tetanus. There is no fever and consciousness is preserved.

An antitoxin which it is stated has therapeutic value in botulism has been

prepared in the usual way by Kempner. Without serum treatment death occurs in about 40% of cases and takes place between twenty-four and forty-eight hours.

The bacillus has been isolated from sausage and ham. It is a large bacillus—5 to 10μ×1μ. It is slightly motile and stains by Gram. It produces gas in glucose

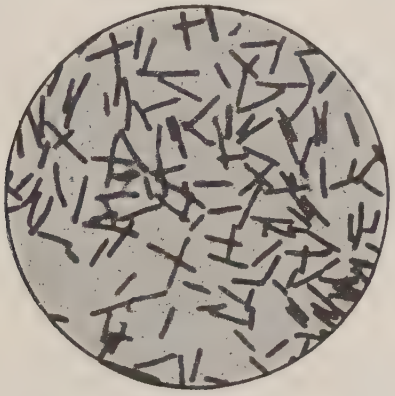

Fig. 21.—Bacillus of botulism, (*Kolle and Wassermann.*)

Fig. 22.—Symptomatic anthrax (Rauschbrand) bacilli showing spores. (*Kolle and Wassermann.*)

media. It grows best at 22° and only slightly at 37°—hence it is dangerous only from its soluble toxin, the bacilli not developing to any extent in the body.

For this reason botulism patients are not a source of danger, it is the infected meat alone which causes the disease. On the contrary where the meat poisoning is due to the Gärtner or paratyphoid group infection may take place from the patient's discharges.

When the toxin is introduced, it requires a period of incubation of twelve to twenty hours. Symptoms of gastrointestinal disorder may come on shortly after the ingestion of the toxin containing food, these however are not the specific manifestations, as are the eye symptoms, etc.

An important point is that ham may not appear decomposed and yet contain many bacilli and much toxin. It is a very potent toxin—as little as one-thousandth of a c.c. may kill a guinea-pig. In man the toxin is apparently absorbed from the alimentary canal. For diagnosis inject an infusion of the ham or sausage which was eaten of into a guinea-pig, and characteristic pupillary symptoms with death by cardiac and respiratory failure will result.

Cultures may be made in glucose agar.

The culture is disrupted by gas. Incubation at room temperature and in the dark is necessary. There is a rancid odor. The characteristic point is the production of a powerful soluble toxin which produces symptoms when no bacilli are present.

B. tetani (Nicolaier, 1885; Kitasato, 1889).—This is the most important organism of the anaerobic spore bearers. Its characteristics are the tetanic symptoms produced by the toxin and the strictly terminal drum-stick spores. Spores are difficult to find in material from wounds infected with tetanus, but readily develop in cultures. Prior to the formation of spores the organism is a long thin bacillus ($4 \times 0.4\mu$). It is motile and Gram positive. It liquefies gelatin slowly and does not coagulate milk.

Theobald Smith recommends growing it in fermentation tubes containing ordinary bouillon, but to which a piece of the liver or spleen of a rabbit or guinea-pig has been introduced at the junction of the closed arm and the open bulb. By this method spores develop rapidly in from twenty-four to thirty-six hours. Sporulation is most rapid at 37° C. As there is always liability to postmortem invasion of viscera by ordinary saprophytes, Smith recommends that great care be taken not to handle the animal roughly in chloroforming and in pinching off pieces of the organ at autopsy. The animal must be healthy, and the tubes to which the piece of tissue is added must be proven sterile by incubation. Smith calls attention to the uncertainty of the temperature at which tetanus spores are killed. He shows that some require temperature only possible with an autoclave. In view of the danger of tetanus, it is advisable to carefully autoclave all material going into bacterial vaccines, such as salt solution, bottles for holding, etc.

Tetanus seems to grow better in symbiosis with aerobes; hence a lacerated dirty wound with its probable contamination with various cocci, etc., and its difficulty of sterilization, offers a favorable soil. The tetanus bacillus gives rise to one of the most powerful poisons known;

it is a soluble toxin like diphtheria toxin, and it is estimated that 1/300 of a grain is fatal for man.

Rosenau has established an antitoxin unit for tetanus which has the power of neutralizing one thousand minimal lethal doses. Practically, it is ten times the least quantity of antitetanic serum necessary to protect the life of a 350 grams guinea-pig from a test dose of tetanus toxin furnished by the hygienic laboratory. (The necessity of some definite unit is apparent when tests have shown that serum stated to contain six million units per c.c. only had a value of 90 of the official American units.) Consequently it is a unit ten times as neutralizing as the diphtheria antitoxin one. The antitoxin of tetanus is less efficient than that of diphtheria for the following reasons:

1. There is about three times as great affinity *in vitro* between diphtheria toxin and antitoxin as is the case with tetanus.

FIG. 23.—Tetanus bacilli showing end spores. (*Kolle and Wassermann.*)

2. The tetanus toxin has greater affinity for nerve cells than for antitoxin.

3. Treatment with antitoxin is successful after symptoms of diphtheria appear. With tetanus it is almost hopeless after the disease shows itself. Hence the importance of the early bacteriological examination of material from a suspicious wound (rusty nail).

4. The tetanus toxin ascends by way of the axis cylinder, and the antitoxin being in the circulating fluids cannot reach it, whereas with diphtheria both toxin and antitoxin are in the circulation. Diphtheria also selects the cells of parenchymatous and lymphatic organs which are more tolerant of injury than the nerve cells. The dose of tetanus antitoxin as a prophylactic is 1500 units; as a curative agent 5000 to 20,000 units. Recent experience shows that it should be injected intravenously when symptoms have manifested themselves.

That the disease is due to toxin is shown not only experimentally, but also if spores are carefully freed of all toxin by washing, and then introduced they do not cause tetanus—the polymorphonuclears engulfing them. The importance of the

presence of ordinary pus cocci in a tetanus wound may be that the activity of the leucocytes in phagocytizing them allows the tetanus bacillus to escape phagocytosis. This would also explain the importance of necrotic tissue in a lacerated wound—the phagocytes taking this up instead of tetanus bacilli. The toxin is digested by the alimentary canal juices and infection by that atrium is improbable. The infection occurs especially through skin wounds, and also from those of mucous membrane. While tetanus is like diphtheria, a disease in which the bacilli are localized and do not spread, yet recently Richardson has obtained tetanus bacilli in pure culture from the tributary lymphatic glands of a "rusty nail" wound of foot. The cultures inoculated into root of tail of a white rat caused the rat's death in forty-eight hours with typical "seal gait" attitude of tetanus in rats.

FIG. 24.—B. aerogenes capsulatus agar culture showing gas formation. (*Williams.*)

The usual period before symptoms occur is fifteen days. The shorter the period of incubation, the more probably fatal the disease. The horse is the most susceptible animal, next the guinea-pig, then the mouse. Fowls are practically immune.

In examining for tetanus, scrape out the material from the suspected wound with a sterile Volkmann spoon and put it in a tube containing blood-serum. Place this in an incubator. We have here the principle of the septic tank—the cocci and other aerobes grow luxuriantly and enable the tetanus bacillus to develop. From day to day smell the culture, and if an odor similar to the penetrating, sour, foul smell of the stools of a man who has been on a debauch be detected, it is suspicious. The nondevelopment of a foul odor is against tetanus. Also make smears from the material and examine for drum-stick spores. If these are found, heat the material to 80° C. for one-half hour, to kill nonsporing aerobes and facultative anaerobes, and then inoculate a deep glucose agar tube

and cultivate by Wright's method. The fusiform lateral outgrowth about the middle of the stab is characteristic.

A more rapid method is to draw up the material, provided it be pus (tissue scrapings may be emulsified in sterile salt solution) into a capillary bulb pipette. Then seal off the end and heat the capillary bulb pipette and its contents in a water bath at 80° C. for 15 minutes. Next break off the sealed tip and stick the pipette into a deep tube of glucose agar. When the point reaches the bottom, force out the material along the line of the stab as the pipette is withdrawn. Cover the surface of the agar with sterile liquid petrolatum and incubate. Better anaerobic conditions obtain where the Buchner or Wright method is employed.

Tetanus produces no gas. Material for examination is best obtained with a bulb pipette (containing a little sterile salt solution) which is plunged into the agar and the salt solution forced out and drawn in where a proper growth is noted.

Spores form in thirty-six to forty-eight hours. In injecting test animals it is advisable to divide the material to be injected into two portions; one animal is injected with the material alone, the second animal with tetanus antitoxin at the same time the material is injected. Only the first animal dies with tetanic symptoms.

B. aerogenes capsulatus (Welch, 1891).—This bacillus is apparently widely distributed. It is possibly the same organism as Klein's B. enteritidis sporogenes, which is constantly present in fæces. It is a large capsulated organism, which does not form chains. Spores are produced on blood-serum. These are frequently absent on other media. It is questioned whether its pathogenicity is other than exceedingly feeble, the presence of the bacillus in emphysematous findings at postmortem being attributed to terminal or cadaveric invasion.

Cases, however, in the Philippines, have been reported following carabao horn wounds, in which most serious and fatal results attended emphysematous lesions showing this bacillus. The isolation of a Gram positive bacillus from a lacerated wound discharge, even in the absence of emphysema, is almost diagnostic.

In milk cultures we have coagulation and from the subsequent development of gas the disruption of the coagulum into shreds. An odor of butyric acid is developed.

Cultures in litmus milk show these shreds plastered against the sides of the tube and showing a pink color.

It is the cause of "foamy organs" occasionally present at autopsy.

The best method of diagnosis is to inoculate the culture or material into the ear vein of a rabbit, kill it and then incubate the body at 37° C. Gas is generated in the organs in a few hours.

Achalme isolated a large bacillus from a fatal case of rheumatism which is now considered as having no relation to acute rheumatism and which was probably B. aerogenes capsulatus.

Kendall has called attention to the importance of this organism in a certain proportion of cases of summer diarrhœa of infants. (See under chapter on fæces.)

CHAPTER VII.

STUDY AND IDENTIFICATION OF BACTERIA. MYCOBACTERIA AND CORYNEBACTERIA. KEY AND NOTES.

Key for Bacilli.—Having branching characteristics, as shown by parallelism, branching, curving forms, V-shapes, clubbing at ends, segmental staining, etc.

Acid-fast. Mycobacterium. { Cultures more or less wrinkled and dry. / More like moulds.

I. Grow rapidly on ordinary media at room temperature.
Examples: Timothy grass bacillus of Moeller (B. phlei).
Mist bacillus. Butter bacilli as reported by (1) Rabinowitsch and (2) Petri.

II. Only grow at about incubator temperature. Scanty growth or none at all on ordinary media. Media of preference are: (a) solidified blood-serum, (b) glycerine agar, (c) glycerine potato and (d) egg media.

1. Cultures fairly moist, luxuriant, and flat. Op. temp. 43° C.
a. Bacillus of avian tuberculosis.

2. Cultures scanty, wrinkled, and dry. Appear in ten to fourteen days. Op. temp. 38° C. Bacilli longer, narrower, more regular in outline and staining than bovine; vacuolation more marked (2.5μ). Smear from organs of inoculated guinea-pig shows few bacilli. Less virulent for rabbits.
a. Bacillus of human tuberculosis.
Cultures as above, but even more scanty. Bacilli shorter, thicker, less vacuolated (1.5μ). Smear from organs of guinea-pig shows many bacilli.
b. Bovine tubercle bacilli.

3. Very difficult to cultivate (Czaplewski).
Smegma bacilli of various animals.

III. Noncultivable by ordinary methods. Cultivable in symbiosis with amœbæ. (Clegg.) Duval cultivated an acid-fast bacillus on N.N.N. medium containing 1% glycerine. Bayon cultivated on placental juice glycerine agar a slightly acid-fast diphtheroid which changed to acid fast in peritoneum of mouse. Bayon's organism thought to be similar to Kedrowsky's diphtheroid of leprosy.

1. B. lepræ. Found chiefly in nasal mucus and in juice from lepra tubercles. Less often in nerve leprosy.

Nonacid-fast. Corynebacterium. { Colonies more flat and moist. Like other bacteria.

I. Do not stain by Gram's method.

1. B. mallei (Glanders). Characteristic culture is that on potato. Growth like layer of honey by third day. Becomes darker in color, until on eighth day is reddish-brown or opaque with greenish-yellow margin.

II. Gram positive.

1. Very luxuriant growth on ordinary media. Colonies often yellow to brownish. B. pseudodiphtheriæ. Shorter, thicker and stain uniformly.
2. Moderate growth on ordinary media. B. diphtheriæ. Best media are blood-serum (Löffler's) or glycerine agar. Has metachromatic granules at poles.
3. Scanty and slow growth on nutrient media. B. xerosis.

The Group of Acid-fast Branching Bacilli.

There is a large and ever-increasing number of organisms which have the same staining reactions as the tubercle bacilli, but which differ in four important essentials of:

1. Growing readily on any media.
2. Showing more or less abundant growth or colonies in twenty-four hours.
3. Having no pathogenic power for guinea-pigs when inoculated subcutaneously.
4. Not requiring body temperature for development, but growing at room temperature.

Many of these organisms, if injected intraperitoneally into guinea-pigs will produce a peritonitis with false membrane. Some also produce granulation tissue nodules which may be confused with true tubercles. For this reason it is well to study the lesions in experimental tuberculosis in the guinea-pig. Injected subcutaneously, on either or both sides of the posterior abdomen with the needle pointing toward the inguinal glands, we may have caseation and ulceration at the site of inoculation. The glands in relation enlarge and caseate. Smears from these show T. B. The marked and characteristic change is the enormous enlargement of the spleen, which is studded with grayish and yellow tubercles. Make smears and cultures from the spleen. The death of the guinea-pig usually occurs in about two months. The lesions may be looked for at three to five weeks.

These nonpathogenic acid-fast bacilli are of greatest importance by reason of their possible confusion with the true tubercle bacilli. Their colonies correspond more or less with different types of tubercle bacilli colonies, being either dry and wrint kled like human, or moist and irregularly flat as avian. Eventually the mois-colonies become dry and wrinkled. They have been isolated from:

1. Butter and milk.
2. From grasses, especially in timothy grass infusion.

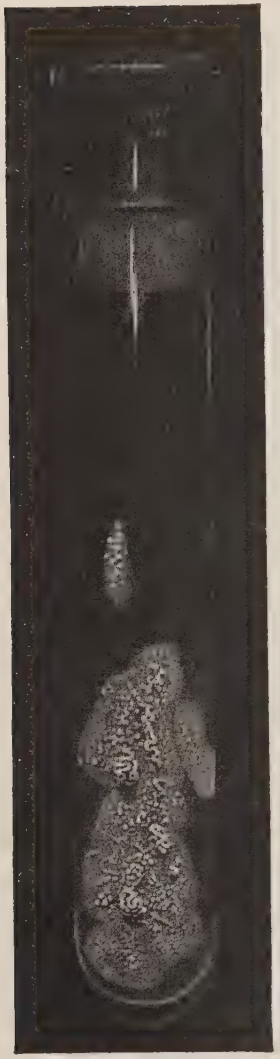

FIG. 25.—Bacillus tuberculosis; glycerine agar-agar culture, several months old. (*Curtis.*)

3. In various excretions of animals, as in dung, urine, etc.
4. Normally in man—from skin, nasal mucus, cerumen, and tonsillar exudate.

It is important to remember that such organisms have very rarely been reported from pulmonary lesions, and when present they have been considered as probably causative.

The present view is that the finding of tubercle bacilli in sputum has practically as great value as it had before we knew of these various acid-fast bacteria.

Tubercle Bacillus (Koch, 1882).—This is a rather long, narrow rod, $3 \times 0.3\mu$. In the human type it tends to show a beaded appearance, this not being due to spores, however. In the bovine type the staining is more solid, the organism shorter and thicker, and shows even a more scanty growth than human T. B. It has been established that many of the tuberculous affections of man, especially those of the skin, bone, and mesenteric glands, are of the bovine type, while, as a rule, pulmonary and laryngeal lesions are of the human type. Experiments by various commissions in different countries have shown that human and bovine types are very closely related and that not only may a bovine strain affect man, but that human T. B. may infect young calves. As bacilli of the bovine type have frequently been reported in intestinal and mesenteric tuberculosis of children it shows the importance of sterilizing cows' milk. Koch considers human infection from bovine sources as of very rare occurrence.

Although Kossel has found only two cases of bovine T. B. in 709 cases of pulmonary tuberculosis yet for the other types the findings are different. Leaving out of consideration the frequency of infections with bovine T. B. in children, recent statistics have shown that in adults about 4% of cervical adenitis, 22% of tabes mesenterica and 3.5% of bone and joint tuberculosis are due to bovine strains of T. B.

The British Royal Commission in its final report of July, 1911, considered three types of T. B.

I. The bovine type belonging to the natural tuberculosis of cattle.

II. The human type. The type more generally found in man.

III. The avian type, belonging to natural tuberculosis of fowls.

The bovine type grows slowly on serum and at the end of two to three weeks shows only a thin grayish uniform growth which is not wrinkled and not pigmented. The human type grows more rapidly and tends to become wrinkled and pigmented. Subcutaneous inoculation of 50 mg. of culture into the neck of calves produced generalized tuberculosis. A similar injection of human T. B. does not cause generalized tuberculosis but only an encapsulated local lesion.

Intravenous injection of 0.01 to 0.1 mg. of bovine T. B. into rabbits causes general miliary tuberculosis and death within five weeks. With human T. B. in doses of 0.1 to 1.0 mg., similarly injected, the majority of rabbits live for three months.

Subcutaneous injection of 10 mg. bovine T. B. causes death in 28 to 101 days. Similar injection of human T. B. in doses up to 100 mg. did not kill the rabbits after periods of from 94 to 725 days. The duration of life in injected guinea-pigs is longer with human than with bovine T. B.

Subcutaneous injections of bovine T. B. into cats produces generalized tuberculosis while the cat is resistant to human T. B. thus given.

Recent statistics (Beitzke) show tuberculous lesions in 58% of adults at autopsy—Naegli's figures were about 90%.

It is a question whether the avian type is absolutely distinct; many experiments having indicated the impossibility of infecting fowls with human T. B. Nocard, by inserting collodion sacs containing bouillon suspensions of human T. B., claims to have changed these to the avian type. The avian type grows at 43° C. fairly luxuriantly, as a moist, more or less spreading culture. It grows much better on glycerinated agar than on serum. Morphologically they are like the human type, but show less tendency to form compact masses. Very pleomorphic. Have been reported from sputum of man (doubtful).

Fowls become infected by intravenous or subcutaneous injection or as the result of feeding. After feeding the lesions are chiefly of the alimentary tract; after injections, of spleen, liver and lungs. Avian T. B. is more virulent for rabbits than human T. B. but less so than bovine T. B. The mouse is the only animal besides the rabbit in which avian T. B. can cause a generalized tuberculosis. The conclusions are that there is no danger to man from avian T. B. With the bovine type it is quite different as nearly one-half of the deaths in young children from abdominal tuberculosis were due to bovine T. B. and to that type alone. Not only in children, but in adolescents suffering from cervical gland tuberculosis, a large proportion were caused by bovine types. The bovine type is also an important factor in lupus.

There is also a fish tuberculosis. This organism grows much more

rapidly than the other types (three to four days), and grows best at 24° C., growth ceasing at 36° C. The colonies are round and moist.

It is certain that many of the symptoms usually noted in the tuberculous are due to secondary infections. Pettit, by careful blood cultures, obtained the pneumococcus in 24 cases and the streptococcus in 36 cases out of 130 cases studied. He used from 5 to 20 c.c. of blood from the vein. Positive blood cultures were obtained in 68% of far-advanced cases, 45% of advanced cases and 16% of incipient cases.

The best culture medium for primary cultures is blood-serum or, better, a mixture of yolk of egg and glycerine agar. Dorset's egg medium is also used. In subcultures, either glycerine agar, glycerine potato, or glycerine bouillon make good media. In inoculating media from tuberculous material, as, say, from a tuberculous gland or, more practically, from the spleen of a guinea-pig, the material must be thoroughly disintegrated or rubbed on the surface of the media so that individual bacilli may rest on the surface of the culture media. In growing in flasks in glycerine bouillon a surface growth is desired. The cylindrical flask of Koch gives a better support to the pellicle than an Erlenmeyer one. In inoculating, a scale of such a surface growth or a grain from the growth on a slant should be deposited on the surface of the glycerine bouillon in the flask.

Inasmuch as the filtrate from cultures has little toxic effect, the poison is assumed to be intracellular.

Koch's old tuberculin, which was simply a concentrated glycerine bouillon culture, is now principally used in veterinary diagnosis. It was prepared as follows:

After four to six weeks the surface growth begins to sink to the bottom of the flask. This fully developed culture is evaporated over a water bath at 80° C. to one-tenth the original volume. It is then filtered, the final product containing about 40% of glycerine.

Koch's tuberculin "R" or new tuberculin was introduced in 1897. In this, virulent bacilli are dried *in vacuo*, ground up in water and centrifuged. The first supernatant fluid (T. O.) is discarded. Subsequent trituration and centrifugalization, preserving each time the supernatant suspension, gives the new tuberculin. It has been found at times to contain virulent T. B.

Koch's bazillen emulsion has been more recently introduced by Koch (1901).

This is simply a suspension of ground up bacilli in 20% glycerine solution. Another preparation is the bouillon filtrate of Denys. This is the unheated filtrate of broth cultures of human T. B. It contains 1/4% phenol.

In the use of T. R. and of bazillen emulsion, Sir A. Wright recommends doses of 1/4000 of a milligram, and he rarely goes beyond 1/1000 of a milligram in treatment. These products come in 1 c.c. bottles containing 5 mg. of bacillary material. It is convenient to remove 2/10 of a c.c., containing 1 mg. Add this to 10 c.c. of glycerine salt solution with 1/4% of lysol. Each c.c. contains 1/10 mg. One c.c. of this stock

solution added to 99 c.c. of salt solution, with 1/4% of lysol, would give a working solution, each c.c. of which would contain 1/1000 mg. of tuberculin.

For diagnostic reactions we have, besides the method of injecting tuberculin and noting presence or absence of fever, six more recent diagnostic tuberculin tests: 1. Variations in opsonic index. 2. Instillation into one eye of a drop of 1/2% or 1% solution of purified tuberculin. Reaction is shown by redness, especially of inner canthus, in twelve to twenty-four hours (Calmette). A previous instillation may sensitize a nontuberculous case and a second application of the drop may give an erroneous diagnosis. 3. The cutaneous inoculation method (similar to ordinary vaccination methods). Scarify two small areas on the arm (1/10 inch in diameter), about 2 inches apart. Rub in one a drop of old tuberculin, in the other a drop of 25% tuberculin. As a control scarify a spot midway and to one side of the others and rub in one drop of 0.5% carbolic glycerine. The appearance of bright red papules in twenty-four hours indicates reaction (von Pirquet). This is the method of preference. 4. Intracutaneous inoculation of one drop of a 1–1000, 1–100 or 1–10 dilution of old tuberculin (Mantoux and Moussu). Webb recommends hypodermic needle points which have been dipped in old tuberculin and the points allowed to dry. A drop of water is placed on the skin and the needle points having been moistened in it are plunged through the skin and withdrawn with a twist. A definite lump shows a positive reaction. 5. Ointment tuberculin test. Rub in 50% ointment of tuberculin in lanolin. Reaction is shown by dermatitis with reddened papules in twenty-four to forty-eight hours (Moro). 6. Inoculation of bovine and human tuberculin to diagnose type of infection (Detre). Of questionable value.

Ebright injects the suspected material into the subcutaneous tissue of one side of the abdomen of three guinea-pigs. At the end of one week an injection into the other side of the abdomen of one of the guinea-pigs of 1/4 c.c. tuberculin is given. Twenty-four hours later smears are made from the original site of inoculation and examined for tubercle bacilli. If negative this is repeated with a second guinea-pig at the end of the second week and finally at the end of the third week with the third guinea-pig.

Bloch's method is to damage the lymphatic glands in the inguinal region by squeezing the tissue between the fingers. Injections made there of tuberculosis material show abundant tubercle bacilli in these damaged glands in ten to twelve days.

In staining it is better to use the Ziehl-Neelsen method, decolorizing with 3% hydrochloric acid in 95% alcohol. The alcohol, for all prac-

tical purposes, enables us to eliminate the smegma and similar bacilli, these being decolorized by such treatment. There are two objections to the Gabbett method, where decolorizer and counterstain are combined: 1. We cannot judge of the degree of decolorization—we are working in the dark; and 2. the matter of elimination of smegma bacilli is impossible.

Pappenheim's method, in which corallin and methylene blue are dissolved in alcohol, does not appear to have an advantage over acid alcohol. As a practical point when the question of tuberculosis of the genito-urinary tract is involved, inoculate a guinea-pig with urinary sediment.

It must be remembered that in young cultures of tubercle bacilli many of the rods are nonacid-fast, taking the blue of the counterstain, while older rods are acid-fast. This frequently causes suspicion of a contaminated culture.

Discussion has arisen as to the granules of Much. These are considered by Much as resistant forms while others consider them degeneration forms of tubercle bacilli. At any rate material containing only these Gram positive granules and no acid-fast rods may when injected into animals give rise to tuberculosis and acid-fast bacilli.

The combination of the acid-fast and Gram staining methods as recommended by Fontes is very satisfactory.

Bacillus Lepræ (Hansen, 1874).—This is the cause of leprosy. In nodular leprosy the organism is readily and in the greatest abundance found in the juice of the tubercles of the skin, and secretions of ulcerations of nasal and pharyngeal mucosa.

The earliest lesion is probably a nasal ulcer at the junction of the bony and cartilaginous septum. Scrapings from this ulcer may give an early diagnosis.

In the skin they are chiefly found in the derma packed in the so-called lepra cells. The process is granulomatous but does not show the caseation of tuberculosis or the predominant plasma cells of syphilis. The bacilli are also found engulfed in the endothelial cells lining the lymphatics.

They are also found in the glands in relation to the superficial lesions. The bacilli are found in smaller numbers in the liver and spleen. In anæsthetic or nerve leprosy they are found in small numbers in the granuloma tissue which affects the interstitial connective tissue of the peripheral nerves. Also, rarely, in the anæsthetic spots of nerve leprosy.

In morphology and staining reactions they are almost identical with the tubercle bacillus. The main points of distinction are: 1. The fact of the leprosy bacilli being found in enormous numbers, especially in large vacuolated cells (lepra cells), and lying in the lymph spaces. They are frequently beaded and lie in masses which have been likened to a bundle of cigars tied together, so that smears show the bacilli in

prodigious numbers. It may be necessary to examine for long periods of time, smears made from tuberculosis lesions of skin before finding a single organism. 2. Leprosy bacilli have not been cultivated with absolute certainty 3. Injected into guinea-pigs, they do not produce lesions.

There have been many reports of positive findings with the Wassermann test in cases of tubercular leprosy but such reports are considered doubtful by many. Butler, in the Philippines, has found that the lepers gave no higher percentage of positive Wassermann reactions than did the nonleprous native patients at his clinic.

Recently a leprosy-like disease of rats has been reported in which there are two types: 1. A skin affection and 2. a glandular one. In this disease, acid-fast bacilli, alike in all respects to leprosy bacilli, have been found. Deane has obtained a diphtheroid-like organism in culture, which is nonacid-fast. This same finding has been obtained in cultures considered positive in human leprosy.

Quite recently it has been claimed that the leprosy bacillus has been cultivated by excising aseptically the subcutaneous portion of lepromata and dropping the leprous tissue into salt solution, the resulting growth being like a streptothrix. This was the basis of the Nastin treatment which is now more or less discredited. In 1909 Clegg reported that by smearing plates containing amœbæ with spleen pulp of lepers (in which the bacilli were abundant) he obtained growth of an acid-fast bacillus. He was able to carry on these organisms in subculture for several generations. A vaccine made from these bacilli does not seem to have been successful.

Duval states that he has cultivated the lepra bacillus on Novy-MacNeal media to which 1% glycerine had been added. He states that white mice can be inoculated and a pure culture obtained from the peritoneal cavity. According to Duval it grows best at 32° to 35° C. and is not killed by a temperature of 60° C. It is most easily obtained by injecting white mice intraperitoneally with material from leprous tissues.

Bayon considers the cultures of Duval and Clegg as not shown to have characteristics which would separate them from the saprophytic group of acid-fast organisms. He thinks that Kedrowsky's nonacid-fast diphtheroid is one stage in the typical acid-fast leprosy bacillus. He states that sera of lepers showed the complement fixation test with antigen made from cultures isolated by himself as well as with the Kedrowsky culture, which tests were negative with Duval's culture.

For diagnosis we should use both smears from the nasal mucus and

from ulcerated lepromata or from the scrapings from intact tubercles. Some advise centrifuging with salt solution, but this is rarely necessary.

The most practical method is by taking a capillary bulb pipette which has been drawn out into a fine point. The point is broken off as with a Wright's blood sticker and inserted deep into the corium. The serum which results is drawn up, smeared out on a slide and stained. The best method is to excise a small portion of skin or mucous membrane, fix it in absolute alcohol or Zenker's fluid. Cut thin sections in paraffin. Stain with carbol fuchsin, decolorize with acid alcohol, and then stain with hæmatoxylin. This gives the location of the bacilli. This is also a good method for tuberculous tissues. It is claimed that the B. lepræ stains more easily and loses its color more rapidly than the tubercle bacillus. Some prefer to stain the leprosy bacillus by Gram's method, it as well as the tubercle bacillus being Gram positive.

Nonacid-fast Branching Bacilli.

Bacillus mallei (Löffler and Shutz, 1882).—This is the cause of a rather common disease of horses. When affecting the superficial lymphatic glands, it is termed "farcy;" when producing ulceration of nasal mucous membrane, the term "glanders" is used.

In man there are two types of glanders—chronic and acute. In the chronic form an abrasion becomes infected from contact with glanders material and an intractable foul discharging ulceration results. This may persist for months with lymphatic involvement or may become acute. The acute form may also develop from the start and the cases are usually diagnosed as pyæmia. Death invariably results in acute glanders. The bacillus is a narrow, slightly curved rod, about $3 \times 0.3\mu$. It is nonmotile and Gram negative. It at times presents a beaded appearance. In subculture on agar or blood-serum the growth is somewhat like typhoid but more translucent. In original cultures from pus or tissues the colonies may not show themselves for forty-eight hours.

As the organism does not tend to invade the blood stream, blood cultures are apt to be negative. The glanders bacillus grows best on an acid glycerine agar (+ 2).

The characteristic culture is that on potato. Grown at 37° C., we have a light brown mucilaginous growth, which by the end of a week spreads out and takes a café au lait color. The potato assumes a dirty-brown color. This and the inoculation of a guinea-pig are the chief diagnostic measures. If the material is injected intraperitoneally into a male guinea-pig, marked swelling of the testicles is noted within

forty-eight hours, at the earliest, to seven to ten days. Cultures should be made from this swollen testicle as other organisms than glanders may bring it about.

Only the B. pyocyaneus and cholera vibrios give a similar coloration of potato. These organisms, however, are easily differentiated. The glanders bacillus is the most dangerous of laboratory cultures and should be handled with extreme care.

The best stains are carbol thionin and formol fuchsin. In sections stained with carbol thionin the bacilli are apt to be decolorized by the subsequent passage of the section through alcohol and xylol. This may be avoided by blotting carefully after the thionin, then clearing with xylol or some oil and mounting. Nicolle's tannin method is a good one.

Mallein is prepared by sterilizing cultures that have grown in glycerine bouillon for about a month by means of heat (100° C.). The dead culture is then filtered through a Berkefeld filter and the filtrate constitutes mallein. It is chiefly used as a means of diagnosing the disease in horses. The reaction consists in rise of temperature and local œdema. The dose is about 1 c.c.

Agglutination and complement fixation tests are also used for diagnosing glanders.

Bacillus diphtheriæ (Klebs discovered, 1883; Löffler cultivated, 1884).—The diphtheria bacillus is found not only in the false membrane which is so characteristic of the disease, but may be found in abundance in the more or less abundant secretions of nose and pharynx. In studying the epidemiology of diphtheria, especial attention must be given to the examination of nasal discharges.

Infection of the larynx and middle ear are not very rare. The mucous membrane of the vagina or the conjunctiva may also be infected. The B. diphtheriæ may be in pure culture lying entangled in the fibrin meshes or contained within leukocytes in the membrane or be associated with staphylococci, pneumococci, or especially streptococci. These latter complicate unfavorably and cause the suppurative conditions about the neck. In fatal cases the diphtheria bacillus may be found in the lungs. Ordinarily, however, it remains entirely local and does not get into the circulation or viscera.

It produces soluble absorbable poisons which are designated toxin in the case of the one responsible for the acute intoxication, parenchymatous degeneration and death; and toxone for the poison which produces œdema at the site of inoculation and postdiphtheritic palsy. The injection of the soluble poisons alone without the bacilli produces the symptoms of the disease.

The bacilli tend to appear as slightly curved rods, showing varying irregularities in staining, as banding or beading, and in particular the

presence at either end of small, deeply staining dots (metachromatic granules). These granules may be seen in an eighteen-hour culture, but within thirty-six hours, may be abundant.

These are well seen with Löffler's blue, but better with Neisser's method. In culture they also show swelling at one or both ends or clubbing. In secretions or in culture they show V-shapes or false branching and, what is most characteristic, the parallelism—four or five bacilli lying side by side like palisades. Being a Gram

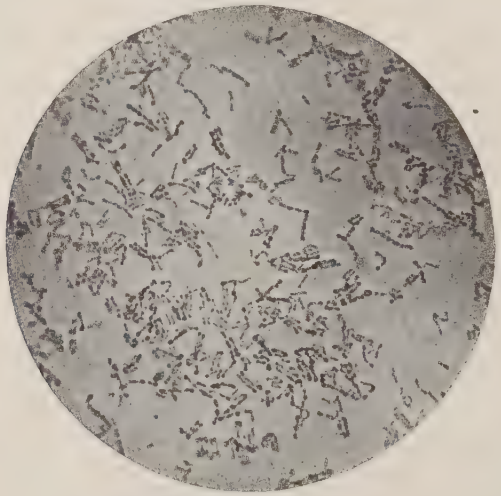

FIG. 26.—Bacillus of diphtheria. (× 1000.) (*Williams.*)

positive organism while the majority of the other pathogenic bacilli are Gram negative, it is of greatest importance to stain smears by this method. It is not so strongly tenacious of the gentian violet as the cocci, so decolorization should not be carried too far.

The best medium for growing it is Löffler's blood-serum.

An egg medium, made of the whole egg with glucose bouillon as described previously, is as suitable as Löffler's serum. Coagulated white of egg answers fairly well, as will a hard-boiled egg—the shell at one end being cracked and the white cut with a sterile knife. This smooth side is then inoculated and the egg placed cut side downward in a sherry glass. If an incubator is not at hand a tube may be carried next the body in a pocket. The bacillus grows better on glycerine agar than on plain agar. On such plates they appear as small, coarsely granular colonies with a central dark area. In size the colonies resemble the streptococcus. On blood-serum the colonies are larger—1/12 to 1/8 inch in diameter.

The diphtheria bacillus grows luxuriantly on blood agar and like the streptococcus pyogenes has a yellowish laked zone around the colony. The Hofman and the Xerosis bacillus do not seem to have this hæmolytic power. In bouillon it tends

to form a surface growth. It is at the surface that the toxin function is most marked, hence in growing diphtheria for toxin formation we use Fernbach flasks which expose a large surface to the air. It is a marked acid producer—bouillon with a +1 reaction becoming +2.5 to +3 in thirty-six hours. The filtrate from a two- or three-weeks-old broth culture is highly toxic, and is usually referred to as diphtheria toxin. It is used in injecting horses to produce antitoxin. Ehrlich uses as a standard to measure the toxicity of toxin the minimal lethal dose (M. L. D.). This is the amount of toxin which will kill a 350-gram guinea-pig in just four days. Some toxins have been produced whose M. L. D. was 1/500 c.c., so that 1 c.c. of such toxin would kill 500 guinea-pigs. Theoretically, the measure of an antitoxin unit is the capacity of neutralizing 200 units of a pure toxin. (On exposure to light, etc., toxin loses

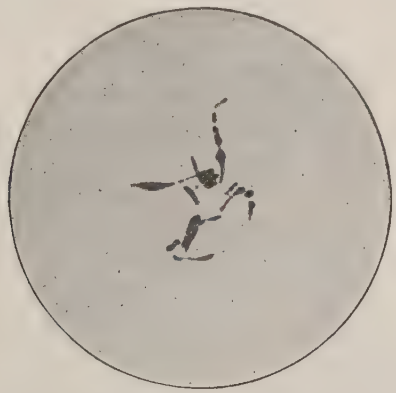

FIG. 27.—Diphtheria bacilli involution forms. (*Kolle and Wassermann.*)

its toxic power and is termed toxoid.) Inasmuch, however, as toxone and toxoid are also present, we may practically consider an antitoxin, or immunizing unit (*i.e.*, Immunitätseinheit) as about capable of making innocuous 100 M. L. D.

In the preparation of antitoxin horses are employed; the method being to inject the bouillon filtrate or toxin subcutaneously at weekly intervals for a period of three or four months. When each c.c. of the serum of the horse is found to contain about 250 to 500 antitoxin units the horse is bled from the jugular vein. Some sera contain as much as 1300 units in a cubic centimeter.

Methods of purifying and concentrating antitoxin are now employed by certain makers, the principle being that the antitoxin in the horse serum is precipitated with the globulins which come down on half saturation with ammonium suplhate. In this way, as the content in horse serum proteids is lessened, the anaphylactic dangers are lessened.

As a curative measure, from 2500 to 5000 units should be injected. If the injection is delayed or the case very serious the dose should be 10,000 units. As much as 50,000 units has been given in severe cases. The prophylactic dose is 500 units.

Sudden death after administration of antitoxin has been reported in cases of status lymphaticus. (See anaphylaxis).

In obtaining material from a throat, be sure that an antiseptic gargle has not been used just prior to taking the throat swab. The part of the swab which touched the membrane or suspicious spot should come in contact with the serum slant. This is best accomplished by revolving the swab. An immediate diagnosis is possible in probably 35% of cases by making a smear from a piece of membrane. In doing this Neisser's stain or the toluidin blue stain are usually considered the most satisfactory. I prefer the Gram stain, however. The diphtheria bacilli found in such smears are not apt to be clubbed and stain more uniformly.

If there is any doubt about the nature of an organism in a throat culture, always stain: 1. with Löffler's alkaline methylene blue for two minutes; 2. with Gram's method, being careful not to carry the decolorization too far, and 3. by Neisser's method. With Löffler's you obtain a picture which, after a little experience, is

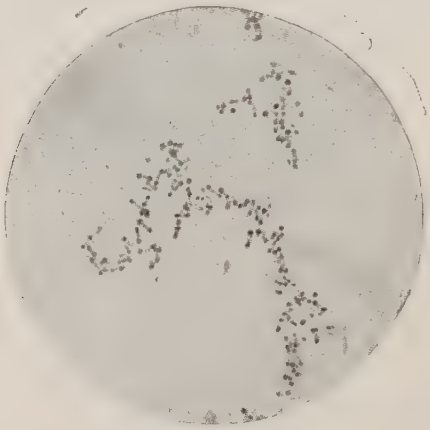

Fig. 28.—B. diphtheriæ stained by Neisser's method. (*Williams.*)

characteristic; at times the polar bodies show as intense blue spots in the lighter blue bacillus. One is liable to confuse cocci lying side by side for diphtheria bacilli with segmental or banded staining. This difficulty is not apparent when Gram's staining is used. This gives us great information, as the diphtheria and the pseudo-diphtheria are the only small Gram positive bacilli usually found in the mouth. The cocci are also well brought out. Neisser's stain gives a picture which, when satisfactory, is almost absolutely characteristic. You have the bright blue dots lying at either end of the light brownish-yellow rods. When first isolated from a throat, the diphtheria bacillus is apt to stain characteristically by Neisser. Later

on, in subculture, there may be no staining of the polar bodies. Neisser originally recommended five seconds' application, with an intermediate washing, for each of his two solutions. Thirty seconds for each is probably preferable. Some authorities recommend five to thirty minutes. It is well to bear in mind that about 2% of the people in apparent health carry diphtheria bacilli of the granular or barred type in their throats and of these about one in five will prove virulent for the guinea-pig.

It is essential when a question exists as to the nature of a diphtheria-like organism to test it as to virulence. While there are exceptions, especially in freshly isolated colonies, yet as a rule a severe infection yields virulent organisms and *vice versa*. Pure cultures are best obtained by streaking material from the throat on glycerine agar plates. From an isolated colony inoculate a tube of bouillon. From such a twenty-four-hour-old culture inoculate a guinea-pig with two or three drops subcutaneously in the shaven abdomen. Escherich considers a fatal result with 1.5 c.c. of such a bouillon culture a satisfactory test as to virulence. After death, which occurs in two or three days, the adrenals are enlarged and hæmorrhagic.

Diphtheroid Bacilli. Pseudodiphtheria Bacillus. Hofman's Bacillus.—Under these terms various Gram positive bacilli have been described as occurring in nose and in skin diseases.

Their chief importance is in connection with their presence in the throats of healthy people. Probably approximately 10% of people harbor such organisms as against 1 to 2% with granular types. Some authorities believe it possible for these diphtheroids to be capable of being transformed into virulent diphtheria bacilli. This seems improbable. Such organisms are often found in urethral discharges, either alone, or with gonococci or other organisms.

1. They very rarely give the blue dot staining at the two ends. Exceptionally they may give a dot at one end. Neisser attaches importance to the dots at both ends as showing diphtheria.
2. They tend to stain solidly or at most with only a single unstained segment. They are shorter, thicker, and do not curve so gracefully as the true diphtheria bacillus. They are stockier.
3. They produce very little acid in sugar media, not one-half that produced by true diphtheria.
4. They are nonpathogenic for guinea-pigs.
5. Many of them grow quite luxuriantly and often show chromogenic power.

Xerosis Bacillus.—This organism is frequently found in normal conjunctival discharges. There is question as to its pathogenesis, and the finding of this organism should not exclude the previous presence of

strictly pathogenic organisms, such as the gonococcus or the Koch-Weeks. It resembles the diphtheria bacillus in being Gram positive and showing parallelism, but differs 1. in being nonvirulent for guinea-pigs; 2. in requiring about two days for the appearance of colonies; 3. in not showing Neisser's granule staining, and 4. in producing very little acid in sugar media.

CHAPTER VIII.

STUDY AND IDENTIFICATION OF BACTERIA. GRAM NEGATIVE BACILLI. KEY AND NOTES.

KEY to the recognition of nonspore-bearing, nonchromogenic, non-Gram-staining, nonbranching bacilli.

(NOTE.—Some books say that the proteus group is Gram positive. It is, however, usually negative.)

Do not grow on ordinary media. Require blood agar (hæmophilic bacteria), serum agar, or blood-serum.

Minute dew-drop colonies.

1. Influenza bacillus. Requires blood media.
2. Koch-Weeks bacillus (conjunctivitis). Serum agar best medium.
3. Müller's bacillus of trachoma. Like Koch-Weeks bacillus, but easier to cultivate.
4. Morax diplobacillus of conjunctivitis. Grows well and produces little pits of liquefaction in Löffler's blood-serum.
5. Bordet-Gengou bacillus of whooping-cough. Does not grow on Löffler's serum. Requires blood or ascitic fluid agar.
6. Ducrey's bacillus (soft chancre). Requires almost pure blood. Forms chains.

Grow well on ordinary media.

I. Cultures in litmus milk. PINK.

A. Nonmotile.

Lactis aerogenes group. B. lactis aerogenes.

Produce gas in glucose, lactose, and saccharose. No liquefaction of gelatin. Short, stubby bacteria, often showing capsules. Intermediate between the colon and Friedländer group.

B. Motile.

1. Nonliquefaction of gelatin.
   a. B. coli group. Coagulation of milk. No subsequent peptonization. Gas in glucose and lactose, none in saccharose. Indol produced. Neutral red reduced.
2. Liquefaction of gelatin.
   a. B. cloacæ group. Gas in glucose, slight in lactose. Slow coagulation of milk. Subsequent peptonization.

II. Cultures in litmus milk. LILAC.

A. Nonmotile bacilli.

1. No gas generated in glucose or lactose bouillon.
   a. Hæmorrhagic septicæmia group. These are oval bacilli with tendency to bipolar staining.

Colonies smaller and less opaque than those of B. coli.
Examples: B. pestis, B. suisepticus, B. choleræ gallinarum (chicken cholera).
B. pseudotuberculosis rodentium (very similar to plague).
B. pestis is absolutely nonmotile, does not liquefy gelatin, does not produce indol, produces slight acid in glucose but not in lactose bouillon.

b. Dysentery group. Colonies similar to those of B. coli.
Divided into two classes according as mannite is acted on:
Those not giving acid—nonacid group—(Shiga-Kruse).
Those giving acid—acid group—(Flexner-Strong).

2. Gas generated in glucose bouillon not in lactose.

a. Friedländer group. Give very viscid, porcelain-like colonies.
Tendency to capsule formation in favorable media.
Examples: B. pneumoniæ, B. capsulatus mucosus, B rhinoscleromatis.

B. Motile bacilli.

1. Do not liquefy gelatin.

a. Do not produce gas in either glucose or lactose bouillon.
Typhoid, or Eberth group. No indol. No coagulation of milk. No reduction of neutral red.

b. Gas generated in glucose, not in lactose media. Milk not coagulated. Neutral red reduced.
Gärtner group. This includes:
Pathogenic types for man; as B. enteritidis, B. icteroides, B. paratyphoid B, B. psittacosis. Nonpathogenic for man; as B. choleræ suum (hog cholera).

2. Liquefy gelatin.

a. Proteus group. Colonies at first round later amœboid, spreading. Produce gas in glucose, not in lactose. Produces foul odor.
B. zopfii type of Proteus group does not liquefy gelatin; colonies at first round, later amœboid, spreading. Foul odor in cultures. Gelatin stab shows lateral branching.

Note.—The Friedländer and the lactis aerogenes group, differing culturally chiefly in carbohydrate fermentation activities, organisms considered as belonging to the Friedländer group rather than to the lactis aerogenes group may show acid in litmus milk. Where an organism having the characteristics of B. coli, but fermenting saccharose, is found, it is termed B. coli communior. A non-gas producing colon type organism has been designated B. coli anaerogenes. Certain organisms which turn litmus milk lilac and which liquefy gelatin, but do not produce gas in sugar media, belong to the "Booker" group. Other organisms which acidify and coagulate litmus milk but do not liquefy gelatin or produce gas in glucose or lactose media have been placed in the "Bienstock" group. The proteus or Hauser group is composed of organisms showing various functions; Proteus vulgaris liquefying gelatin rapidly, P. mirabilis slowly and P. zenkeri not at all.

Gram Negative Bacilli Requiring Special Media.

Bacillus influenzæ (Pfeiffer, 1892).—This organism is the type of the so-called hæmophilic bacteria—organisms whose growth is restricted

to media containing hæmoglobin. The influenza bacillus seems to grow better on slants freshly streaked with blood than on those which have been made for some time, and they appear to grow better on this surface smear of blood than on a mixture of agar and blood.

The influenza bacilli are most likely to be isolated from the sputum of bronchopneumonia due to this organism. It has also frequently been found in the nasal secretions of influenza patients. Exceptionally, it is present in the blood, and has been isolated in cases of meningitis from cerebrospinal fluid. It also occurs at times in anginas, but then usually associated with other organisms. Infection probably only takes place by contact. It is a very small bacillus which in sputum tends to show itself in aggregations, especially centering about M. tetragenus. It stains rather faintly when compared with cocci, so that a smear of sputum stained with formol fuchsin shows a deep violet staining for the M. tetragenus or other cocci, and scattered around in a clump-like aggregation we see these minute, rather faintly stained rods. They also tend to stain more deeply at either end, so that they sometimes appear as diplococci. Gram's method, counterstaining with formol fuchsin, is excellent for their demonstration. The red bacilli and the violet-black cocci are easily distinguished.

To cultivate them, rub the sputum, or at autopsy the material from a lung, on a slant smeared with human blood (pigeon's blood is also satisfactory), and then without sterilizing the loop, inoculate a second blood slant; then a third, and possibly a fourth. The colonies appear as very minute dewdrop-like points which seem to run into each other in a wave-like way. To test such colonies we should transfer a single colony to plain agar and blood-serum, trying not to carry over any blood. If the least trace of blood is carried over, they may grow on agar or blood-serum. Organisms resembling the influenza bacillus have been isolated from whooping-cough. Such organisms have also been found in the fauces of well persons. In many epidemics of influenza the bacillus has not been isolated, or success has obtained in only a small proportion of the cases. Etiological factors in conditions more or less resembling influenza may be the Streptococcus, Pneumococcus, or M. catarrhalis. The influenza bacillus seems to grow best in symbiosis with some other organism, especially with S. pyogenes aureus.

Koch-Weeks Bacillus (Koch, 1883).—This produces a severe conjunctivitis. It is very common in Egypt and is also a frequent cause of conjunctivitis in the Philippines and in temperate climates.

Smears made from conjunctival secretion show large numbers of small Gram-negative bacilli, especially contained within pus cells, but also lying free. They are more difficult to cultivate than the influenza bacillus, but the same general methods hold. The vitality of this

organism is very slight so that almost immediate transferrence of material is necessary. Flies are an important factor in Egypt. The period of incubation is short, twelve to thirty-six hours. The best medium is a mixture of glycerine agar and hydrocele or ascites fluid. At first we rarely obtain pure cultures. The colonies are dewdrop-like and first show themselves in about thirty-six hours in incubator cultures.

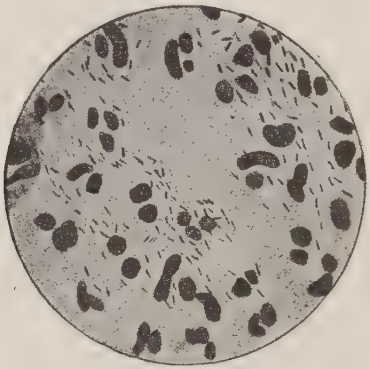

FIG. 29.—The Koch-Weeks Bacillus. (*Hansell and Sweet.*)

Diplobacillus of Morax.—This organism causes mild blepharo-conjunctivitis chiefly at the internal angle of the eye. They are about 1 or 2μ long and tend to occur in pairs or short chains. Some claim that they are Gram positive.

Culturally the formation of little pits of liquefaction in Löffler's serum within twenty-four hours which later become confluent may be regarded as fairly characteristic. They do not grow on nutrient agar.

After two or three days on blood-serum rather marked involution forms occur. While usually causing a more or less chronic conjunctivitis they may at times produce a keratitis.

Bacillus of Chancroid (Ducrey, 1889).—These are short coccobacilli, occurring chiefly in chains. They show bipolar staining. They grow best in a mixture of blood and bouillon.

Bacillus of Bordet-Gengou.—This bacillus was reported as the cause of whooping-cough by Bordet and Gengou in 1906. (Czaplewski and Reyher had previously reported oval bipolar staining organisms, as the cause of pertussis, and other authors influenza-like organisms.)

The bacillus is oval, Gram negative, shows bipolar staining, somewhat resembles B. influenzæ and grows only on uncoagulated serum media, as blood or ascites agar. The original cultures are very scanty so that the colonies are difficult to recognize. In subcultures the growth is more flourishing. The organism is only found in white, thick, leukocyte abounding sputum, of the beginning of the disease. Hence pertussis is probably contagious only at the onset.

Complement binding and agglutination reactions have been obtained. For diagnosis stain the sputum. Remember that pertussis gives a mononuclear leukocytosis of 15 to 50 thousand.

Gram Negative Bacilli Growing on Ordinary Media.

Bacillus pneumoniæ (Friedländer, 1882).—This organism is responsible for about 5% of the cases of pneumonia. It is usually termed the pneumobacillus to distinguish it from the pneumococcus; at other times Friedländer's bacillus. The name of Fraenkel attaches to the pneumococcus. Morphologically, it is a short, thick bacillus, and in pathological material, as sputum, shows a wide capsule. It is nonmotile and Gram negative. The colonies on agar are of a pearly whiteness and are markedly viscid. On potato it shows a thick viscid growth containing gas bubbles. The characteristic culture is the nail culture of a gelatin stab. The growth at the surface is heaped up like a round-headed nail, the line of puncture resembling the shaft of the nail. It does not liquefy gelatin. It does not produce indol, and does not produce gas in lactose bouillon—differences from the colon bacillus—with which it may be confused in cultures, as it does not then possess a capsule. If in doubt, inject a mouse at the root of the tail. Death from septicæmia occurs in two days. The peritoneum is sticky and numerous capsulated bacilli are present in the blood and organs. The organisms which have been isolated from rhinoscleroma and ozœna are practically identical with the B. pneumoniæ. This group of organisms is generally referred to as the Friedländer group. Similar organisms have been isolated from the discharges of middle-ear diseases and in anginas. Cases have been reported where the B. pneumoniæ was the cause of septicæmia in man.

Bacillus pestis (Kitasato, Yersin, 1894).—This is the organism of plague. It is primarily a disease of rats. It is the member of the group of hæmorrhagic septicæmias (Pasteurelloses), from which man suffers.

Other Pasteurelloses are chicken cholera, swine plague, mouse septicæmia and rabbit septicæmia. This is a widely distributed group and may include saprophytic organisms as well as those noted for their virulence.

B. choleræ gallinarum and B. suisepticus are approximately similar in size and cultural requirements to B. pestis. The oval bacillus with bipolar staining in smears from tissues is very characteristic for both of them. Another name for swine plague (B. suisepticus) is infectious pneumonia of swine. The organism is chiefly found in the lungs.

Where the plague bacilli are found chiefly in the glands, we have bubonic plague; when in lungs, pneumonic plague; when localized in the skin and subcutaneous tissue, the cellulo-cutaneous; and when as a septicæmia, septicæmic plague. An intestinal type is recognized by

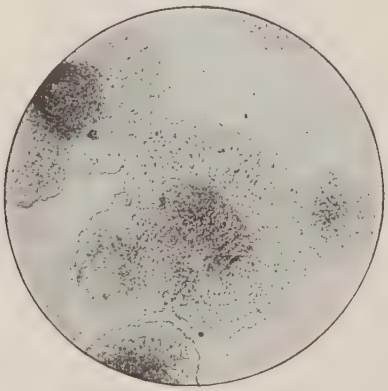

FIG. 30.—Colonies of plague bacilli forty-eight hours old. (*Kolle and Wassermann.*)

some authors. It must be remembered that in all forms of plague the lymphatic glands show hemorrhagic œdema; it is in bubonic plague, however, that the areas of necrosis with periglandular œdema are prominent. Where the symptoms are slight, mainly buboes, the term pestis minor is sometimes used; the typical disease being termed pestis major. In pneumonic plague we have a bronchopneumonia.

In smears from material from buboes, from sputum, or in blood smears, as well as from blood or spleen smears from experimental animals, we obtain the typical morphology of a coccobacillus ($1.5 \times 0.5\mu$) with very characteristic bipolar staining; there being an intermediate, unstained area. Very characteristic also is the appearance in these smears of degenerate types which stain feebly and show coccoid and inflated oval types. The presence of these involution forms associated with typical bacilli is almost diagnostic for one with experience. Inoculating tubes of plain agar and 3% salt agar with this same material, we obtain in plain agar cultures organisms which are typically small, fairly slender rods, which do not stain characteristically at each end and are not oval. The smear obtained from the salt agar presents most remarkable involution forms—coccoid; root-shaped, sausage-shaped forms, ranging

from three to twelve microns in length, more resembling cultures of moulds than bacteria. Another point is that on the inoculated plain agar we are in doubt at the end of twenty-four hours whether the dewdrop-like colonies are really bacterial colonies or only condensation particles. By the second day, however, these colonies have an opaque grayish appearance, so that now, instead of questioning the presence of a culture, we consider the possibility of contamination.

Blood cultures in septicæmic plague may show from 5 to 500,000 per c.c. Smears from the blood in such cases are positive in only about 17%.

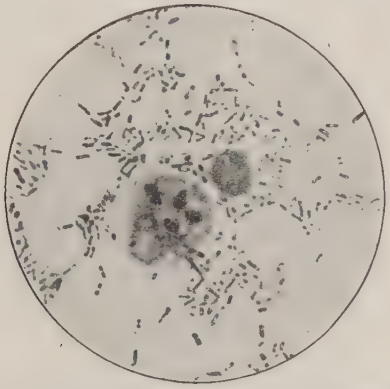

FIG. 31.—Pest bacilli from spleen of a rat. (*Kolle and Wassermann.*)

The plague bacillus grows well at room temperature—its optimum temperature being 30° instead of 37° C., as is usual with pathogens. Next to the salt agar culture, the most characteristic one is the stalactite growth in bouillon containing oil drops on its surface. The culture grows downward from the under surface of the oil drops as a powdery thread. These are very fragile, and as the slightest jar breaks them, it is difficult to obtain this cultural characteristic.

While Klein states that B. coli, proteus vulgaris and, in particular, B. bristolensis may be mistaken for plague bacilli, if bipolar staining alone be relied upon, yet it is B. pseudotuberculosis rodentium which may confuse an experienced worker. While this latter is only moderately pathogenic for rats yet the fact that rats may be immunized to B. pestis by inoculation with B. pseudotuberculosis rodentium brings up the suspicion of identity of the two organisms. In diagnosing always use animal experimentation. Owing to the difficulty in emulsifying plague bacilli, agglutination tests are not satisfactory.

Albrecht and Ghon have shown that by smearing material upon the intact, shaven skin of a guinea-pig, infection occurs. This is the most crucial test.

A pocket made by cutting the skin of a guinea-pig with scissors and extended subcutaneously with scissors or forceps, into which a piece of the suspected plague tissue is thrust with forceps, is more practical than injecting an emulsion with hypodermic syringe.

Mice inoculated at the root of the tail quickly succumb. Rats, this being primarily a disease of rats, are of course susceptible. Other rodents, as squirrels, are susceptible. It has been suggested that a rodent, the Siberian marmot, or tarabagan (Arctomys bobac) might be the starting-point of plague outbreaks. In natural plague of rats, the lesions which establish a diagnosis even without the aid of a microscope are dark red, subcutaneous injection of the flaps of the abdominal walls as they are turned back, fluid in the pleural cavities, œdematous hæmorrhagic

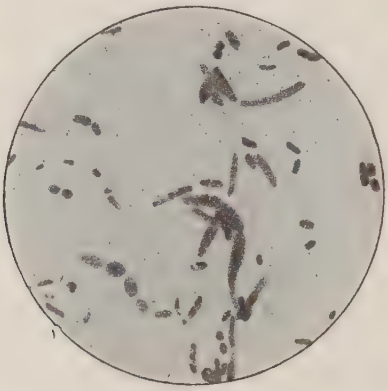

FIG. 32.—Pest bacillus involution forms produced by growing on 3% salt agar. (*Kolle and Wassermann.*)

periglandular infiltration and swelling of the neck glands, and in particular a creamy, mottled appearance of the liver. The neck glands are chiefly involved because the flea prefers to inhabit the skin of the neck. Smears from the spleen will show the oval bacilli.

A chronic rat plague, which may be a factor in keeping up the disease, is characterized by enlargement of the spleen and the presence within it of nodules containing plague bacilli. McCoy has noted that the frequency of the cervical bubo in rats, noted by the Indian Commission (72%), was not found in California. The glands show periglandular infiltration and injection as well as enlargement.

Recent investigations in India have definitely determined the fact that the flea (Xenopsylla cheopis) is the intermediary in the transmission of plague from rat to rat and from rat to man. In primary pneumonic plague the infective nature is very great and appears to be by the respiratory atrium (From man to man). This was the terrifying type of plague in the black death of the fourteenth century.

Strong and Teague have shown that of 39 plates exposed before the mouths of patients with pneumonic plague, with marked dyspnœa and pulmonary œdema, but without coughing, only one plate showed plague bacilli. In 39 other experimental plate cultures with coughing on the part of the patients there were 15 plates showing plague bacilli.

The droplet method of infection is therefore the important one in plague pneumonia.

As these droplets are expelled to a considerable distance not only should the respiratory inlets be protected by masks but the conjunctivæ with glasses and abrasions with protective coatings.

For diagnosis make smears and cultures from material drawn from a bubo by a syringe. (At a later stage, when softening begins, there may not be any bacilli present.) Also, if pneumonic plague, from the sputum. Blood cultures and even blood smears may be employed in septicæmic plague. Formol fuchsin and Archibald's stain make satisfactory stains. Always inoculate a guinea-pig with the material either by rubbing it in with a glass spatula on the shaven skin or by subcutaneous injection. For prophylaxis the most important method is that of Haffkine. Stalactite bouillon cultures of plague are grown for five to six weeks. These are killed by a temperature of 65° C. for one hour. Lysol (1/4%) is added to the preparation and from 0.5 to 4 c.c. injected, according to the age and size of the individual treated. Susceptibility is reduced about one-fourth, and of those attacked after previous vaccination, the mortality is only about one-fourth of what it is among the noninoculated. Strong prepares a prophylactic vaccine from living plague cultures rendered avirulent. Yersin's serum, made by injecting horses with dead plague cultures and afterward with living ones, is of value prophylactically and has possibly considerable curative power.

The Eberth, Gärtner and Escherich Groups.—From a standpoint of cultures in litmus milk and sugar bouillon we can divide the organisms related to typhoid at one extreme and the colon at the other into three groups.

1. The Eberth or typhoid group. There are three important pathogens in this group: the B. typhosus, the B. dysenteriæ, and the B. fæcalis alkaligenes. The color of litmus milk is practically unaltered and there is no gas production in either glucose or lactose bouillon. No coagulation of milk. No reduction of neutral red. The B. typhosus and the B. fæcalis alkaligenes are actively motile, while the B. dysenteriæ is nonmotile or practically so.

During the first twenty-four to forty-eight hours there is a moderate acid production by typhoid, so that the milk culture is less blue, while with the B. fæcalis alkaligenes the alkalinity is intensified from the start, so that the blue color is deepened.

2. The Gärtner or hog cholera group. Besides organisms important for animals and probably at times for man, such as B. choleræ suum and B. psittacosis and B. icteroides (interesting historically as having been reported as the cause of yellow fever by Sanarelli), we have two pathogens: 1. B. enteritidis (Gärtner's bacillus) and 2. B. paratyphoid B. In this connection it may be stated that the present view is that hog cholera is caused by an ultra-microscopic organism and not by the B. choleræ suum.

These organisms cannot be separated culturally, but only by immunity reactions. They do not turn litmus milk pink. They produce gas in glucose bouillon, but not in lactose. They very powerfully reduce neutral red with the production of a yellowish fluorescence. They do not coagulate milk. There is a transient acidity in the litmus milk, but becoming shortly afterward alkaline, the lilac-blue color is intensified. Both organisms are motile.

3. The Escherich or colon group. These turn litmus milk pink, coagulate milk, reduce neutral red, and show varying degrees of motility. The three groups of organisms just described are nonliquefiers of gelatin. Two intestinal organisms, the B. cloacæ and the Proteus vulgaris, differ in liquefying gelatin.

Bacillus typhosus (Eberth, 1880; Gaffky, 1884).—This organism may be isolated from the stools, urine, and the blood of typhoid patients.

At postmortem it can be best isolated from the spleen, but is also present in Peyer's patches which have not ulcerated. When ulceration has occurred contamination with B. coli. is almost sure. Cultures may be obtained from the liver also. In sections made from spleen the Gram negative bacilli are apt to be decolorized. Thionin, then blotting and clearing in oil or xylol, shows the clumps of bacilli lying between the cells.

Formerly it was supposed that by the differences in the thickness of the film of a colony or by its varying shades of grayish-blue, we possessed data of importance in differentiating typhoid from related organisms.

The colonies look like grapevine leaves.

Growth on potato was also considered as affording information. At present, the biochemical reactions give us information assisting in differentiation, and the agglutination and bacteriolytic phenomena, the final diagnosis. The various plating media are considered under media for plating out fæces.

Not only do we find hyperplasia of the endothelial cells in the lymphoid tissue

of Peyer's patches and the mesenteric glands and the spleen, with subsequent necroses, but focal necroses of the same character are found in the liver.

A striking feature of the pathology of typhoid fever is the long-continued persistence of the organisms in the gall-bladder and elsewhere. It is beginning to be believed that a previous typhoid infection, possibly so mild as to have passed unnoticed, is at the basis of gall-bladder infections and resulting gall-stones. Various bone infections, especially osteomyelitis, have shown the typhoid bacilli in pure culture. Formerly it was supposed that the typhoid bacillus brought about its lesions by a local infection centered in the ileum. The present view is that typhoid bacilli effect an entrance into the blood stream through some lymphoid channel, as by tonsil or other alimentary lymphoid structure. Of animals, only the chimpanzee seems to be susceptible.

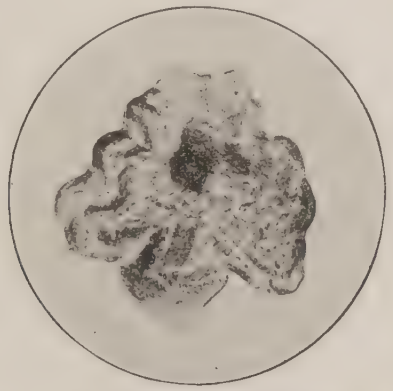

FIG. 33.—Seventy-two-hour-old culture of typhoid bacillus on gelatin. (*Kolle and Wassermann.*)

They develop in the general lymphatic system, the spleen in particular, where they are protected from the bactericidal power of the blood. After a time, however, approximately the period of incubation, they become so abundant in these lymphatic organs that they are carried over into the general circulation. Then as a result of bacteriolysis the intracellular toxins are liberated and symptoms develop. If bacteriolysis takes place other than in the blood we have various suppurative processes. As a result of the formation of antibodies, the development in spleen, etc., is checked but should these immunity reactions become less potent relapses may occur or various local infections manifest themselves.

As the bacilli do not multiply to any extent in the blood itself the disease cannot be considered as a typical septicæmia but as a bacteriæmia.

Typhoid bacilli can be isolated from the blood during the latter period of incubation and rarely after the tenth day of the disease. It is a practical point that the time to isolate the bacteria from the blood is in the first days of the attack. The diagnosis by agglutination is only expected after the seventh to tenth day. Agglutination may not appear until during convalescence, and in about 5% of the cases it is absent. It, as a rule, disappears within a year.

Very little success has been obtained with curative sera. Chantamesse, by treating horses with a filtrate from cultures of typhoid bacilli on splenic pulp and human defibrinated blood, claimed to have obtained a curative serum possessing antitoxic power. Wright's method of prophylactic inoculation is now being employed in the British army with apparent success. In this, twenty-four- to forty-eight-hour-old cultures are killed at 53° C.; 1/4% of lysol is then added. An injection of 500 million bacteria is made at the first inoculation, and ten days later an injection of one billion. The British prefer to inject subcutaneously in the infraclavicular region and at the insertion of the deltoid. The Germans consider three injections as conferring greater immunity.

Russell has obtained splendid results in the U. S. Army with his method of vaccination. In this three injections are given at intervals of ten days, the dosage being 500,000,000, for the first and 1,000,000,000 for each of the two succeeding injections.

Typhoid vaccines sterilized with 0.5% of phenol appear to keep much longer and to have a higher immunizing power than those prepared by sterilization with heat and subsequent addition of the antiseptic.

Typhoid bacilli may be found not only in the blood, urine and fæces but as well in the sputum of cases showing pulmonary involvement. They have also been found in the cerebrospinal fluid of cases showing meningeal symptoms. At the autopsy they may be found in the spleen, Pyer's patches, mesenteric glands and liver.

A very important discovery is that certain persons, who may have had only a slight febrile attack, may eliminate typhoid bacilli for years in their fæces (typhoid carriers). The bacilli are also eliminated for considerable periods in the urine. Distinction is now being made between acute carriers (convalescents) and chronic carriers.

The most satisfactory method of detecting carriers is by examination of fæces or urine plated out on Endo's medium. While carriers usually give a Widal reaction this is by no means constant. Typhoid carriers are said to maintain a high opsonic index.

The urine and fæces of typhoid convalescents should be proven negative by cultural procedure before discharging the patients.

Vaccination seems to be a very satisfactory measure in bringing about the disappearance of typhoid bacilli in the dejecta of carriers.

For laboratory diagnosis, blood cultures during the first week and agglutination tests during the second week and onward are the practical methods.

Along with the agglutination tests the urine and fæces should be cultured on Endo's plating medium and later transferred to Russell's medium for cultural identification. The positive identification, provided the culture so isolated shows the cultural characteristics of typhoid, is made by testing the bacilli for agglutination with a known typhoid serum. Instead of the usual blood cultures one may use the clot in the Wright U-tube for culturing and the serum remaining after centrifugaliza-

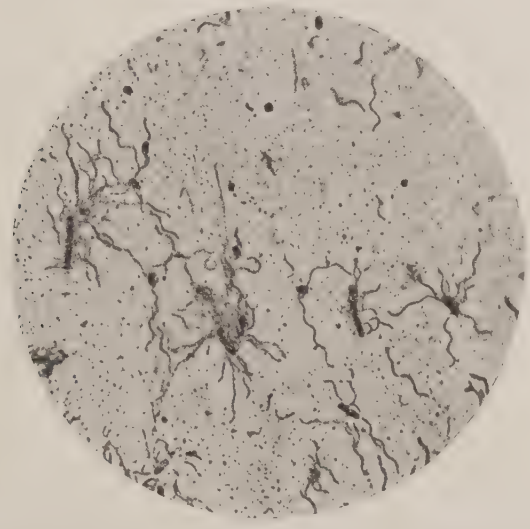

FIG. 34.—Bacillus of typhoid fever, stained by Löffler's method to show flagella. (× 1000.) (*Williams.*)

tion for the Widal test (clot culture). B. typhosus appears in the blood in relapses. Kayser considered that about 27% of cases of typhoid in Strasburg were caused by raw milk, 17% by contaminated water, 17% by contact with typhoid, and 10% were due to typhoid carriers. Other cases were due to infected food, and about 13% were of origin impossible to determine. These latter may have been due to unrecognized typhoid carriers. He does not attach the same importance to fly dissemination as do American authors.

Contact infection is the great factor in perpetuating typhoid fever but this agency shows diminishing cases each year provided water and milk supplies are safe. The leading European cities as a result of a safe

water supply rarely show more than about three typhoid deaths per 100,000 population per year. Edinburgh shows less than one per 200,000 for the year 1910. In American cities rates of twelve to fifteen per 100,000 are common.

The Gärtner or Meat-poisoning Group.—Under this designation may be considered the organisms which cause gastrointestinal disorders of varying degrees, infection with which is usually brought about by the ingestion of meat obtained from diseased cattle. Unless the meat is thoroughly cooked the bacilli in the interior may not be killed.

In this group may be placed B. enteritidis, the typical meat-poisoning organism, B. paratyphoid B, B. Danysz, B. Aertryck, B. typhi murium and B. suipestifer.

B. suipestifer or the hog cholera bacillus was formally thought to be the cause of this important epizootic. It is found in the intestines of quite a percentage of healthy hogs. The cause is now known to be a filterable virus.

These organisms are alike morphologically and culturally and show quite a tendency to bipolar staining and reduction of neutral red with fluorescence in forty-eight hours. B. paratyphoid B, B. Aertryck and B. suipestifer are alike from an agglutination standpoint, while B. enteritidis and B. Danysz show similarity in this respect. B. paratyphoid A stands by itself.

Paratyphoid Bacilli (Achard and Bensaude, 1896; Schottmüller, 1901).—Cases resembling mild attacks of typhoid occasionally show agglutination for paratyphoid bacilli. These organisms have also been isolated from the blood, as with typhoid. Two types have been recognized: the paratyphoid A and the paratyphoid B. The latter occurs in 80% of such cases. Culturally, paratyphoid B. cannot be separated from Gärtner's bacillus. In paratyphoid A there is less gas produced in glucose bouillon than with paratyphoid B, and the primary acidity of litmus milk is not succeeded by a subsequent alkalinity. It does not seem practical to draw a fine distinction between these two strains.

Paratyphoid B. not only gives symptoms resembling a mild typhoid infection, but may show symptoms more like those of meat poisoning or even cholerine. It is more pathogenic for laboratory animals than is B. typhosus. The development of antibodies upon immunizing a man or animal with paratyphoid organism does not seem to approach that obtained with typhoid.

Bacillus enteritidis (Gärtner, 1888).—This organism has been frequently isolated from cases of gastroenteritis from ingestion of infected meat.

Meat from healthy animals which has been in contact with that of diseased animals may become infected. The simple act of placing a piece of infected meat

on a sound piece may infect the latter. It has been noted that the bacteria, or their toxins, may be distributed unevenly in the meat eaten, so that one person consuming the same meat may be made very ill while others eating this meat may escape infection. Infection of food may occur not only from unclean handling but from the material carried by flies or even from the fæces of mice or rats deposited on foodstuffs.

This organism is very pathogenic for laboratory animals, producing a hæmorrhagic enteritis and at times a septicæmia. Where meat has been contaminated with Gärtner's bacillus toxins may have been produced, and symptoms of poisoning with acute gastroenteritis would occur shortly after ingestion. This is not a true toxin as it does not require a period of incubation before manifesting its toxic action. It is interesting to note that this toxin is not destroyed by the boiling temperature, thus differing from the toxin of the other important meat-poisoning (botulism) bacillus—B. botulinus—which is rendered innocuous by a temperature of 65° or 70° C. If there is only a little toxin introduced with the contaminated meat, the symptoms will be delayed one or two days. Such organisms have been isolated in pure culture from cases with high fever, marked intestinal derangement, with considerable blood in the rather fluid stools. In two cases studied the disease was at first diagnosed as a severe typhoid infection. Klein thinks the organism of Danysz's virus (to kill rats during plague epidemics) may be identical with B. enteritidis.

Proteus vulgaris.—This organism is often encountered in plates made from fæces, or sewage contaminated water.

It is common in decaying meat or cheese, and cases of even fatal poisoning with marked gastrointestinal symptoms and cardiac failure have been reported. At times it is the cause of cystitis. The colonies on agar are moist and unevenly spreading (amœboid). The bacilli are very motile, long and slender, tend to form filaments and, as a rule, are Gram negative. It digests blood-serum and is a rapid liquefier of gelatin. In litmus milk it coagulates with a soft clot and an alkaline reaction. Subsequently the litmus is reduced and the clot digested giving a dirty yellowish-brown fluid. Indol is rarely produced. The cultures generally have a putrefactive odor. In infective jaundice (Weil's disease) this organism has been reported as the cause. Organisms of this group were formerly designated as B. termo.

Bacillus dysenteriæ (Shiga, 1898).—Dysentery bacilli produce a coagulation necrosis of the mucous membrane of the large intestine and occasionally of the lower part of the ileum. Polymorphonuclears are contained in the fibrin exudate.

It was formerly thought that these lesions were of local origin, but the present view is that toxins are produced which, being absorbed, are eliminated by the large intestine with resulting necrosis. Flexner, by injecting rabbits intravenously with a toxic autolysate, produced characteristic intestinal lesions. The toxin withstands a temperature of 70° C. without being destroyed. The toxin may cause joint trouble.

There are two main types of dysentery bacilli:

1. Those producing acid in mannite media—the acid strains (Flexner-Strong types).

2. Those not developing acid in mannite (Shiga-Kruse types). Ohno finds that fermentative reactions do not correspond to immunity ones. Thus an acid strain used to immunize a horse may produce a serum more specific for a nonacid strain. The Shiga type is very toxic in cultures, while the Flexner type does not seem to possess a soluble toxin.

The Shiga strains are apt to cause a paresis of the hind extremities of the injected rabbit which may be followed by paralysis and death. At the Lister Institute injections of a soluble toxin produced a serum of marked antitoxic power. Such a dysentery serum, which is probably both antitoxic and antimicrobic, is of curative value. Shiga immunized horses with polyvalent cultures and obtained a polyvalent serum which has reduced the death rate about one-third.

The dysentery bacillus is present in the milky white, leukocyte filled blood flecked mucous stools during the first five or six days of the disease. By the tenth day it has probably disappeared. Lactose litmus agar is the most satisfactory plating medium. The stool of the first two days may give practically a pure culture. The staining of a smear from the muco-purulent stool is rich in phagocytic cells, many of them packed with Gram negative bacilli. In all cultural respects the dysentery bacillus resembles the typhoid, and the only practical method of distinguishing these two organisms, other than by agglutination reactions, is by the nonmotility or exceedingly slight motility of the dysentery bacillus.

The characteristic of nonmotility is of greatest differentiating value and the reports of slight motility are probably from misinterpretation of molecular movement as motility. The dysentery bacilli do not form those threads or whip-like filaments so characteristic of typhoid cultures and are somewhat plumper. The dysentery bacillus is not found in the blood and hence is not eliminated in the urine. It is found in mesenteric glands. In dysentery patients agglutination phenomena do not show themselves until about the twelfth day from the onset. Hence, this procedure is of no particular value in diagnosis. It is of value, however, to identify an organism isolated from the stools at the commencement of the attack, using serum from an immunized animal or a human convalescent for the agglutination test.

Butler has suggested taking serum from dysentery convalescents, noting the strain involved, and preserving it by taking up with filter paper as recommended by Noguchi for the Wassermann hæmolytic amboceptor. This I consider very

valuable as it is very difficult to immunize rabbits with a Shiga strain on account of its great toxicity.

There seems to be very little agglutination power in the serum of convalescents from Shiga strains. Flexner strains give agglutination, but early in convalescence the serum is not apt to have a titre of more than 1–50.

Morgan has reported as the cause of certain cases of bacillary dysentery a bacillus known as *B. Morgan, No.* 1. It is motile, produce indol, and in glucose bouillon gives a very slight amount of gas.

It does not change mannite and does not produce a primary acidity in litmus milk. This organism is a frequent cause of summer diarrhœa of children. Flies from houses with such cases often show Morgan's bacillus. A dysentery type much like the Flexner Strong strain is often found in the enteric affections of children in the United States.

In Japan, dysentery-like epidemics of a very fatal disease, termed *ekiri*, occur among young children. The organism is very motile, producing gas and acid in glucose but not in lactose media. It is reported to at times show indol production. Apparently a member of the Gärtner group.

More recently a strain of dysentery bacilli, known as *Type Y*, has been considered of importance. This organism is very closely related to the Flexner strain and only differs from it in that it requires about 48 hours to turn mannite litmus media pink and that maltose litmus remains blue. An organism showing similar cultural characteristics has been recently recovered from fæces of laboratory rabbits by German workers investigating the problem of whether certain animals might serve as carriers for dysentery.

B. Coli, B. Lactis Aerogenes, B. Cloacæ.

While the COLON BACILLUS chiefly inhabits the large intestine, the B. lactis aerogenes is to be found in the upper part of the small intestine. While they may be separated on the ground of motility, yet it is by the greater fermentative activity of the B. lactis aerogenes that they are best separated. Some consider them as only representing different strains of the same organism. B. lactis aerogenes is closely related to the pneumobacillus and at times shows capsules. Some consider that the B. coli produces a bactericidal substance which inhibits the growth of, or destroys, pathogenic bacteria which may have passed the destructive influences of the gastric juice; others that this effect is due to their free

growth and the development of phenol and various putrefactive substances. The probable importance of the colon bacillus in protecting the organism is shown by the fact that where numerous colonies of pathogenic organisms may be cultivated from fæces we may find a diminution in number or absence of the colon bacillus. This condition may be observed in infections with the organisms of dysentery, cholera, typhoid, and paratyphoid. While its normal function is probably protective, yet the B. coli is an important pathogenic agent, it being frequently the organism isolated from purulent conditions within the abdominal cavity, especially in appendicitis and lesions about the bile ducts. It is particularly prone to cause lesions of the bladder and pelvis of the kidney. In the treatment of colon cystitis by vaccines of dead colon bacilli, the most brilliant results in opsonic therapy have been obtained.

Sir A. Wright thinks that certain cases of mucous colitis may be due to colon infection and that vaccination may cure them. The colon bacillus is fully considered under the bacteriology of water.

B. CLOACÆ was isolated first from sewage by Jordan. It is, as a rule, a rapid liquefier of gelatin, and in its reactions with sugars and litmus milk resembles the colon bacillus.

Where the gelatin liquefaction is slow or slight it may be distinguished from B. coli by its gas formula which is about three times as much CO_2 as H, just the reverse of that of the colon bacillus. B. lactis aerogenes is often found in sewage. It is one of the causes of the souring of milk.

B. Acidophilus, B. Bifidus, B. Bulgaricus.

These are often termed the long rod group of lactic acid bacteria in contradistinction to certain other Gram positive bacilli which are short and oval and which are confused with the so-called milk streptococci.

The long rod group often forms chains and often shows metachromatic granules which stain with Neisser's method. They are readily distinguished from Gram negative lactic acid producers, of which the type is B. lactis aerogenes, by their Gram positive staining. B. acidophilus often give the impression of a diphtheroid in a Gram stained fæces smear. It is nonmotile and often shows polar granules. Grows only at temperatures above 22° C., op. 37° C. It grows better anaerobically than aerobically and then shows the clubbed involution characteristics of B. bifidus; so that some consider these organisms the same, the morphology of B. bifidus being the result of anaerobiosis. Original cultures are best made in 1% glucose and 1% acetic acid bouillon. Some authorities consider B. bifidus the most important

representative of the large intestine flora. B. lactis acidi is less thermophilic than B. acidophilus and coagulates milk which B. acidophilus does not do. Certain polar granule bacteria, as B. granulosum, found in Yoghurt, are similar to B. acidophilus but coagulate milk; no gas. B. bulgaricus is the type of the group and is discussed under milk.

Rodella thinks B. acidophilus, B. bifidus, B. gastrophilus and the Boas-Oppler bacillus identical. B. bulgaricus is said to never show polar granules. B. bulgaricus and the group of organisms similar to it found in buttermilk, etc., are widely used in the treatment of various intestinal troubles. North has used cultures of B. bulgaricus for extermination of undesirable organisms in other parts of the body than the alimentary canal (used as applications in nasal, throat or genito-urinary infections).

Chromogenic Bacilli.

These are identified by the color of their colonies on agar. The B. pyocyaneus is the most important one of them in medicine, but the B. prodigiosus is also of interest medically. A violet chromogen, the B. violaceus, which is motile and liquefies gelatin, has been described under many names. It has been found in water.

An orange-yellow chromogen, the B. fulvus, is nonmotile and varies as to its liquefaction of gelatin.

B. pyocyaneus (Gessard, 1882).—This organism is frequently termed the bacillus of green or blue pus. It is a small ($2.5 \times 0.5\mu$) motile Gram negative bacillus.

It is generally a slender delicate bacillus often showing thread-like arrangement but at times it may appear as short plump rods. It grows readily at room or incubator temperature. It liquefies gelatin rapidly. The green color diffuses through the agar or gelatin on which it grows, so that we not only have the green-colored colony, but the medium as well is colored. Upon potato the colonies are more of a deep olive green to dirty brown.

No gas is produced in either glucose or lactose bouillon; blood-serum is digested, the pitted surface showing a reddish-brown color. The protein ferment pyocyanase has been used to remove diphtheritic membrane and for treatment of M. catarrhalis nasal catarrhs. There are two pigments—a green water soluble one and a blue one soluble in chloroform.

It is widely distributed in water and air, and is frequently isolated from fæces. The B. fluorescens liquefaciens of water seems to be simply a strain of B. pyocyaneus. The B. pyocyaneus is frequently associated with other pus organisms in abdominal abscesses.

In addition to having an endotoxin, it produces a soluble toxin similar to diphtheria toxin. This toxin differs from those of diphtheria and tetanus in that it can stand a temperature of 100° C., while those of diphtheria and tetanus are destroyed at about 65° C. The fact that the union between toxin and antitoxin is only of a binding, neutralizing nature is best shown by taking a mixture of pyocyaneus toxin and antitoxin which is innocuous and heating it. This destroys the antitoxin, but does not injure the toxin. We now find that the original toxicity has returned. The antitoxins of diphtheria and tetanus are more stable than the corresponding toxins; hence, this experiment would be impossible with them, as upon heating we should first destroy the toxin.

On account of the frequent association of B. pyocyaneus with other organisms of better recognized pathogenicity it has until more recently been considered rather harmless; this view can no longer be entertained as it is frequently the sole cause of middle-ear inflammations, intestinal disorders and possibly at times of septicæmia.

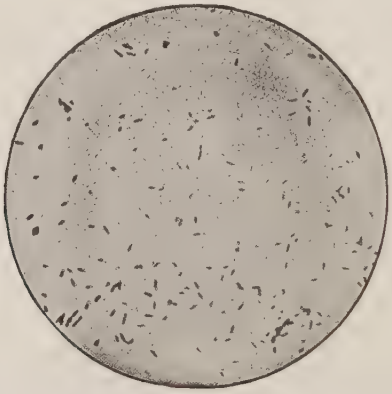

FIG. 35.—Bacillus pyocyaneus. (*Kolle and Wassermenn.*)

B. prodigiosus.—This is a very small coccobacillus which shows motility in young bouillon cultures. It is Gram negative. The colonies on agar or other solid media show a rich red color. The pigment only develops at room temperature; it is absent in cultures taken out of the incubator. The B. prodigiosus is frequently found on foodstuffs, especially bread, where it may simulate blood. It liquefies gelatin rapidly and gives a diffuse turbidity to bouillon. It is probable that B. indicus and B. kilensis are strains of B. prodigiosus.

Coley's fluid, which has been used in cases of inoperable sarcoma and other malignant growths, is a culture prepared by growing very virulent streptococci in

bouillon for ten days. This streptococcus culture is now inoculated with B. prodigiosus, and after another ten days the mixed culture is killed by heat at 60° C. and the sterile product injected. Coley injected about one-twentieth of a c.c. of this vaccine. At present he uses nonfiltered, heat sterilized bouillon cultures of a streptococcus obtained either from a case of erysipelas or septicæmia. To this is added material from agar cultures of B. prodigiosus, grown separately and sterilized before adding to the sterilized streptococcus bouillon culture.

CHAPTER IX.

STUDY AND IDENTIFICATION OF BACTERIA. SPIRILLA. KEY AND NOTES.

KEY to recognition of gelatin liquefying, motile and Gram negative spiral or comma-shaped organisms.

A. Do not give the nitroso-indol reaction with sulphuric acid alone in twenty-four hours.

1. Produce an abundant moist cream-colored growth on potato at room temperature.
   a. Finkler and Prior's spirillum (Vibrio proteus). Liquefaction of gelatin very rapid. No air-bubble appearance at top of liquefied area. Cultures have foul odor. Milk coagulated. Thicker spirillum than cholera. Isolated from cholera nostras.
2. Scanty growth or none at all on potato at room temperature. Only a moderate yellowish growth when incubated about incubator temperature.
   a. Spirillum tyrogenum (Deneke's spirillum). Does not liquefy gelatin so rapidly as Finkler Prior. Thinner and smaller spirillum than cholera.

B. Give the nitroso-indol reaction with sulphuric acid within twenty-four hours.

1. Very pathogenic for pigeons.
   a. Spirillum metschnikovi. Liquefies gelatin about twice as rapidly as cholera. Gives bubble appearance at top of stab.
2. Scarcely pathogenic for pigeons.
   a. Spirillum choleræ asiaticæ.

Nonmotile, nonliquefying and Gram positive spirilla have also been described. There is also a large group of phosphorescent spirilla.

Spirillum choleræ asiaticæ (Koch, 1884).—Typically, the morphology of this organism is that of the comma (Comma bacillus of Koch). It also frequently shows S shapes, and often appears in long threads showing turns. When freshly isolated from cholera material they, as a rule, show a fairly typical morphology, but after subcultures in the laboratory variations are common, so that rod forms and round involution shapes give a picture altogether at variance with the comma shape.

Even in recent cultures of undoubted cholera we may have different types, as coccoid forms and slender rods. Ohno has noted the fact that the same strain of cholera will give at one time vibrio forms and again coccoid or rod forms, depending

on the reaction of the media. Inasmuch as the recognition of vibrio shapes is of importance in diagnosis he recommends that material from a stool be inoculated into three tubes of peptone solution of reaction +0.3, −0.5 and −1, respectively, one of which would probably show vibrio morphology.

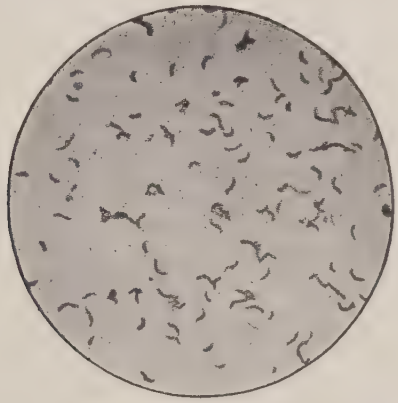

FIG. 36.—Cholera spirilla. (*Kolle and Wassermann.*)

The cholera spirillum is very motile (a scintillating motility) and liquefies gelatin fairly rapidly, although more slowly than any of the spirilla mentioned in the key. The colony on gelatin was formerly considered characteristic, but like most cultural characteristics, it is now

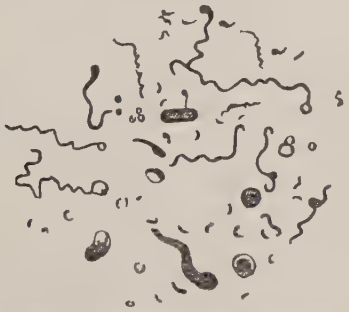

FIG. 37.—Involution forms of the spirillum of cholera. (*Van Ermengen.*)

considered as being only of confirmatory value; it is not specific. These colonies show in twenty-four hours as small granular white spots which have a spinose periphery. An encircling ring of liquefaction now makes its appearance and the highly refractile (as if fragments of spark-

ling glass) colony can be separated into a granular center, a striated periphery, and a clear external ring of liquefaction.

On gelatin stabs the liquefaction produces a turnip-like hollow at the top of the puncture—the air bubble appearance. It gives the nitroso-indol reaction with sulphuric acid alone (cholera red). Kraus attaches importance to the fact that cholera does not produce a hæmolytic ring on blood agar as do the pseudocholera spirilla; a difficulty is that many pseudospirilla do not hæmolize. Furthermore, true cholera strains may occasionally show hæmolysis, especially in laboratory cultures. Quite a discussion has arisen in connection with a spirillum isolated from cases of diarrhœa (no symptoms of cholera) in pilgrims at El Tor. This organism gave the immunity reactions (agglutination) of true cholera but on account of its hæmolytic power has been considered as distinct from cholera. Such a view would seem to be untenable. Sp. choleræ grows very rapidly on peptone solution and this is the medium for the enrichment test to be later described. On this it may form a pellicle. On agar the colony is more opalescent (more of a translucent grayish blue) than the typhoid. It does not grow on potato except at incubator temperature. It does not coagulate or turn acid litmus milk. The spirilla are found in myriads in the rice-water discharges, these white flakes being desquamated epithelial cells. They penetrate the crypts of Lieberkuhn, but rarely extend to the submucosa. The symptoms are due to an endotoxin.

Fig. 38.—Spirillum of cholera stab culture in gelatin two days old. (*Fraenkel and Pfeiffer.*)

Cholera may be transmitted from water supplies, when the outbreak is apt to be widespread and in great numbers from the start. Also by indirect contagion, as by flies or on lettuce, etc. A very important point is that we have well persons whose fæces contain virulent cholera spirilla (cholera carriers).

Cholera spirilla disappear from the stools of cholera patients very rapidly, usually in five to ten days. Cholera carriers are therefore of less importance epidemiologically than typhoid carriers.

It is well to remember however that cases have been reported of positive findings after a period approximating two months from the onset of the attack of cholera. Another important consideration is that the vibrios may be absent at one examination and be present at a later one. Purgatives seem to influence the reappearance of the spirilla. An acid reaction of the fæces, such as that induced by lactic acid bacteria, would apparently be of value in the prophylaxis of cholera carriers.

Greig has found infection of the bile of the gall-bladder or ducts in 80 cases in 271 cholera autopsies. While cholera spirilla are soon crowded out by intestinal bacteria, thus explaining the short period during which cholera spirilla are excreted by convalescents, this is not true when the cholera vibrio gets into the bile ducts or gall-bladder, where ideal conditions prevail for a prolonged life. In fact bile has

recently been recommended as a selective medium for cholera enrichment. Greig found one cholera convalescent excreting cholera vibrios 44 days after the attack. Of twenty-seven persons who had been in contact with cholera patients six were excreting cholera vibrios though apparently well.

To identify such spirilla immunity reactions are necessary:

1. Injected intraperitoneally into guinea-pigs, it produces a peritonitis and subnormal temperature. This reaction exists for spirilla other than the true cholera spirillum.
2. Intramuscular injections into pigeons are only slightly pathogenic, if at all.
3. The agglutination test is the most practical. In this we use serum from an immunized animal, in dilution of from 100 to 1000. It is rare that true cholera vibrios fail to agglutinate in serum of 1 to 500 and even sera of 1 to 10,000 dilution give the reaction. Serum of cholera convalescents may show agglutination as early as the tenth day; it is usually best shown about the third week. Dunbar's quick method is very practical. Make two hanging-drop preparations, using mucus from the stool as the bacillary emulsion. To one add an equal amount of a 1 : 50 normal serum; to the other a 1 : 500 dilution of immune serum. Cholera spirilla remain motile in the control, but lose motility and become agglutinated in the preparation with the immune serum.
4. Pfeiffer's phenomenon. If cholera spirilla are introduced into the peritoneal cavity of immunized guinea-pigs (or if together with a 1 : 1000 dilution of immune serum the mixture is injected intraperitoneally into normal guinea-pigs) and at periods of ten to sixty minutes after injection, material is removed by a pipette from the peritoneal cavity, the spirilla have lost motility, have become granular and degenerated. Pseudospirilla are unchanged. This reaction may be carried on in a pipette, using fresh serum.

Antisera for the treatment of cholera have not proved successful. Prophylactically, there are two prominent methods: 1. That of Haffkine, where live cholera spirilla are injected subcutaneously; and 2. Strong's cholera autolysate. In this cholera cultures are killed at 60° C. The killed culture is then allowed to digest itself in the incubator at 37° C. for three or four days (peptonization). The preparation is then filtered and from 2 to 5 c.c. of the filtrate is injected. Ferran was the first to use vaccines.

For diagnosis: 1. take a fleck of mucus, make a straight smear and fix; stain with a 1 : 10 carbol fuchsin. The comma-shaped organisms appear as fish swimming in a stream.

2. Inoculate a tube of peptone solution. The cholera spirilla grow so rapidly, and being strong aerobes, they grow on the surface of the fluid so that by taking a loopful from the surface, we may in three to eight hours obtain a pure culture. Should there be a pellicle present, this should be avoided in the transfer by tilting the tube slightly, so that the material near the surface be obtained without touching the pellicle. Inoculate a second tube from the surface of this first and, if necessary, a third (enrichment method).
3. Test for cholera red reaction. (Simply adding from three to five drops of concentrated chemically pure sulphuric acid to the first or second peptone culture after eighteen to twenty-four hours' growth. Some specimens of peptone do not give the reaction.) At times we only get the cholera red when we have a pure culture of cholera.
4. Smear a fleck of mucus or, better, the three hour surface growth of a peptone culture on a dry agar surface in a Petri dish. From colonies developing, make agglutination and, if desired, cultural tests. It is by immunity reactions that we identify cholera spirilla. The surface moisture of plates is best dried by the filter-paper top.

The cholera colony is easily distinguished from the ordinary fæcal bacterial colonies by its transparent, bluish-gray, delicate character. It emulsifies with the greatest ease. A practical, quick method is to make smears from suspicious colonies, stain for one minute with dilute carbol fuchsin and if vibrios are present to make two vaseline rings on a single slide allowing ample space at one end for handling the preparation safely. Inside of one ring deposit with a platinum loop a drop of salt solution and inside the ring nearest the end which is to be held by fingers or forceps, deposit a loopful of 1 to 500 or 1 to 1000 dilution of cholera serum. The emulsion in the salt solution remains uniformly turbid and under a low power of the microscope (2/3 in.) shows a scintillating motility. The emulsion made into the drop of serum quickly shows a curdy agglutination and upon examination with the 2/3-in. objective shows clumping and absence of motility. Cover-glasses placed over the two vaseline rings assist in the study of the preparation.

CHAPTER X.

STUDY AND IDENTIFICATION OF MOULDS.

Classification of the Fungi.

Order	Suborder	Family	Genus	Species
Phycomycetes	Zygomycetes		Mucor	M. corymbifer
				M. mucedo
			Rhizomucor	R. septatus
			Rhizopus	R. niger
		Saccharomycetes	Saccharomyces	S. cerevisiæ
				S. anginæ
				S. blanchardi
			Endomyces	E. albicans
			Cryptococcus	C. gilchristi
				C. hominis
Ascomycetes	Gymnoascus	Gymnoasceæ	Trichophyton	T. sabouraudi
				T. tonsurans
				T. violaceum
				T. mentagrophytes
				T. cruris
			Microsporum	M. audouini
			Achorion	A. schoeleini
	Carpoascus	Perisporiaceæ	Penicillium	P. crustaceum
			Aspergillus	A. fumigatus
				A. concentricus
				A. pictor
				A. niger
			Discomyces	D. bovis
				D. maduræ
Hyphomycetes			Madurella	M. mycetomi
			Malassezia	M. furfur
			Microsporoides	M. minutissimus
			Trichosporum	T. giganteum
			Sporotrichum	S. beurmanni

Note.—In many of the works on bacteriology considerable space is given to the so-called Higher Bacteria. The organisms are chiefly considered under the names Leptothrix or forms in which are found simple nonbranching threads, Cladothrix or thread-like forms with false branching and Streptothrix or forms showing true branching. It is not practical to consider any separate group distinct from the so-called Lower Bacteria on the one hand and the Fungi on the other.

The Fungi.

The Thallophyta are plants in which there is no differentiation between root and stem.

The classes of Thallophyta which are of interest medically are 1. the Algæ and 2. the Fungi.

Some include Lichenes as a separate class. These are really symbiotic organisms—Fungi parasitic on Algæ.

The Algæ contain chlorophyll, with the exception of Cyanophyceæ. To the order Cyanophyceæ it is considered that the family of bacteria belong.

The fungi do not possess chlorophyll. They are in their simplest forms ramifying filaments called hyphæ. The vegetative hyphæ which intertwine in tangled threads, as a support, are termed the mycelium, while those which project upward are called the aërial hyphæ and are the ones which bear the conidia or spores.

The aërial hypha which carries the fruiting organ encasing the conidia (sporangium) is called the sporangiophore and the more or less rounded termination of this hypha, which projects into the sporangium, is called the columella.

The hypha may be composed of one cell or of many cells separated by septa (septate).

The orders of the class Fungi which are of interest medically are: 1. the Phycomycetes; 2. the Ascomycetes; 3. the Hyphomycetes.

Phycomycetes.—These produce a copious network-like mycelium, which is non-septate, and reproduce asexually by means of a sporangium, a case-like structure borne on the clubbed extremity of an erect hypha (columella) and containing numerous spores or, as in the case of the suborder Oomycetes, reproduction is by heterogamy. (Dissimilar sexual cells—a smaller male, antheridium, and a larger female, oogonium. By fertilization by antherozoids from the antheridium penetrating the oösphere we have oöspores.)

The suborder Zygomycetes reproduces either asexually (a sporangium filled with spores) or by isogamy (two similar but sexually differentiated cells conjugate and form on fusion a zygospore).

Belonging to this suborder we have four families, only one of which, the Mucor family, is of importance medically. In this family we have three genera: Mucor, without rhizoids; Rhizopus, with rhizoids and unbranched aërial hyphæ and, Rhizomucor, with rhizoids and ramified mycelium.

Two species of Mucor are of pathogenic importance.

1. Mucor mucedo and 2. Mucor corymbifer. These moulds develop especially in external cavities as nasopharynx and external ear.

Pulmonary and generalized infections have also been reported. The pathogenic species have smaller spores and grow best at 37° C. The thick, coarse, cotton-like mould seen on horse manure is a Mucor. The sporangium, the organ of fructifica-

tion, contains the spores within its interior. The M. mucedo has thick silver-gray mycelium, with large sporangia, 150μ in diameter, containing oval spores, 5 × 9μ. The M. corymbifer, which has been reported from a generalized infection, considered as typhoid, shows a snow-white mycelium. The sporangia are 20 to 40μ and the spore about 3μ in diameter.

Rhizopus niger has a columella which becomes distorted into a mushroom shape after the spores have been discharged from the sporangium. This mould has been considered as the cause of a mycosis of the tongue.

Ascomycetes.—In this order are included many of the parasitic moulds. The most distinctive characteristic is the formation of ascospores in an ascus (little sac). It is an enlarged extremity of a hyphal branch in which a definite number of spores, usually eight, is formed. The ascus usually ruptures at its tip. Other members of the order are

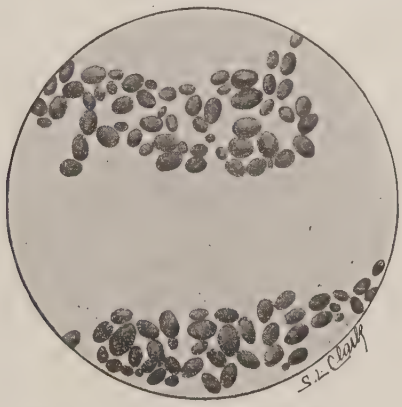

FIG. 39.—Yeast cells. Saccharomyces cerevisiæ. (*Coplin.*)

formed from hyphæ by the separation of cells in succession from the free cells. The mycelium is septate.

The order is divided into those with naked asci (Gymnoascus) and those having a perithecium or investing layer about the ascus (Carpoascus).

Belonging to the suborder Gymnoascus we have 1. the family of Saccharomycetes, which reproduce by budding and in which the asci are without any semblance of a sheath, and 2. a family in which there is an indication of the formation of a perithecium—this may be termed the Gymnoasceæ family.

Saccharomycetes.—There are three genera: Saccharomyces, Endomyces, and Cryptococcus.

Saccharomyces.—These reproduce by budding, have ascopores and no mycelial-like threads.

S. cerevisiæ.—This is the ordinary yeast fungus. Used at times as an antiseptic.

S. anginæ.—Found in a case of angina.

S. blanchardi.—Found in a jelly-like tumor mass of the abdomen. The budding cells varied from 2 to 20μ. Probably identical with S. tumefaciens, reported as the cause of a subcutaneous tumor about region of Scarpa's triangle.

Endomyces.—Forms spores in the interior of filaments, or by ascus formation or by chlamydospores (resistant spore-like structures with a thick membrane which project from the extremities or sides of the hyphæ as bud-like structures).

E. albicans.—The organism of thrush. It produces a false membrane, especially on buccal surfaces, which is easily detached and beneath which the mucosa is intact. Grows only in acid media. Hence propriety of alkaline treatment.

Cryptococcus.—Reproduces by budding, but ascospore formation not observed. Not a well-recognized genus. The diseases caused by it are termed blastomycoses.

C. Gilchristi.—The cells are about 16μ in diameter and have a thick, double contoured membrane. They reproduce by budding. The skin lesions resemble various infectious granulomata and diagnosis rests on the finding of budding

Fig. 40.—Thrush fungus. (*Kolle and Wassermann.*)

or sporulating cells. It may invade internal organs. Original cultures are obtained with some difficulty and then best with Löffler's serum. Subcultures grow readily. Potato is a good medium and on it we may have both mycelial and yeast-like growth. Guinea-pigs can be inoculated subcutaneously. A mould, somewhat similar, is the Coccidioides immitis of Ophuls. This has a mycelial growth in tissues, this distinguishing it from the former fungus. The infection frequently becomes generalized. The small bodies, about 3μ, in the Molluscum contagiosum cells are thought by some to be yeasts. They are more probably artefacts. Plimmer's bodies in cancer cells belong in this group. They also are probably other than parasites.

C. linguæ pilosæ.—This is a more or less elongated yeast-like organism and supposed to be the cause of black tongue, a benign affection of the lingual papillæ.

Gymnoasceæ.—Belonging to the family Gymnoasceæ we have the genera Trichophyton, Microsporum and Achorion.

The trichophytons are generally known as the large-spored ring-worms. The spores are in chains and may be inside the hair or both outside and inside. Many of them are of animal origin, especially from the horse and the cat. The spores are from 5 to 7μ.

The mycelium is greatly segmented, shows simple or dichotomous branching, and produces spores within the mycelium.

T. tonsurans.—Gives a crater-like culture with fine marginal rays. Fungus wholly inside the hair. Causes most of the large-spored scalp ringworms and many body cases.

T. sabouraudi.—Has a heaped-up festooned sort of culture. There is a similar

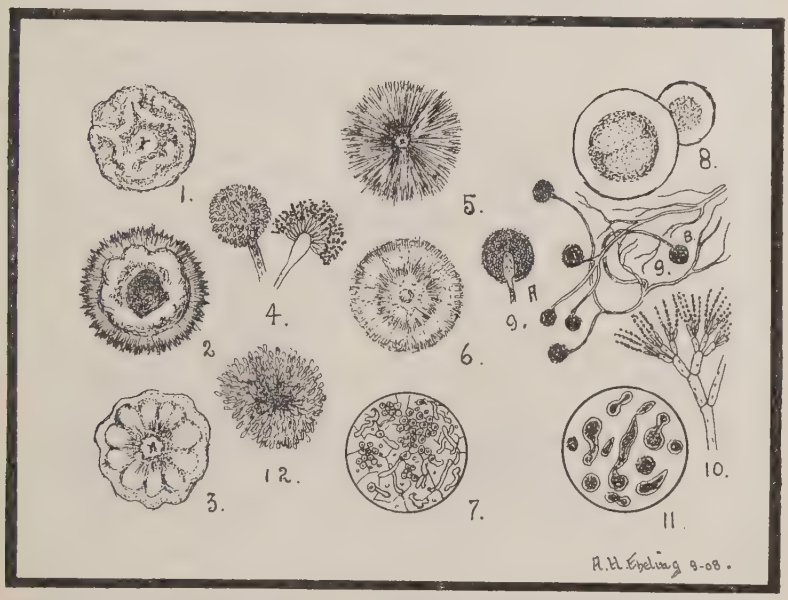

FIG. 41.—More common fungi. 1, Culture of Achorion schoenleini (favus); 2, culture of Trichophyton tonsurans; 3, culture of Trichophyton sabouraudi; 4, sporangium of Aspergillus; 5, culture of Trichophyton mentagrophytes; 6, culture of Microsporum audouini; 7, mycelium and spores of Malassezia furfur; 8, Cryptococcus gilchristi; 9, A and B, sporangium and mycelium of Mucor corymbifer; 10, Penicillium; 11, Saccharomyces tumefaciens; 12, Discomyces bovis.

fungus with a violet culture. These cause some of the scalp and beard ringworms.

T. mentagrophytes.—This is the T. megalsporon endoectothrix of Sabouraud. The external spores are in chains or in short mycelial threads, not mosaics of spores, and are of very unequal size (2 to 11 microns). There are varieties from horse, cat, and bird. The lesions are more inflammatory than those of the endothrix class. Most of the beard and body ringworms belong to this group—very few scalp cases. The cultures are finely rayed.

Some give yellow cultures, others white and one derived from birds a rose-colored culture.

Microsporum audouini.—This is the so-called small-spored ringworm and is a very common and highly contagious affection of the scalp in children in England and France; less so in other countries.

It is almost never seen in the tropics. It almost exclusively affects the hairy scalp. The spores are 2 to 3μ in diameter. The broken stump of the hair is characteristic. The fungus is packed as a mosaic of spores, forming a white sheath, chiefly on the outside of the hairs. It gives a downy-white culture.

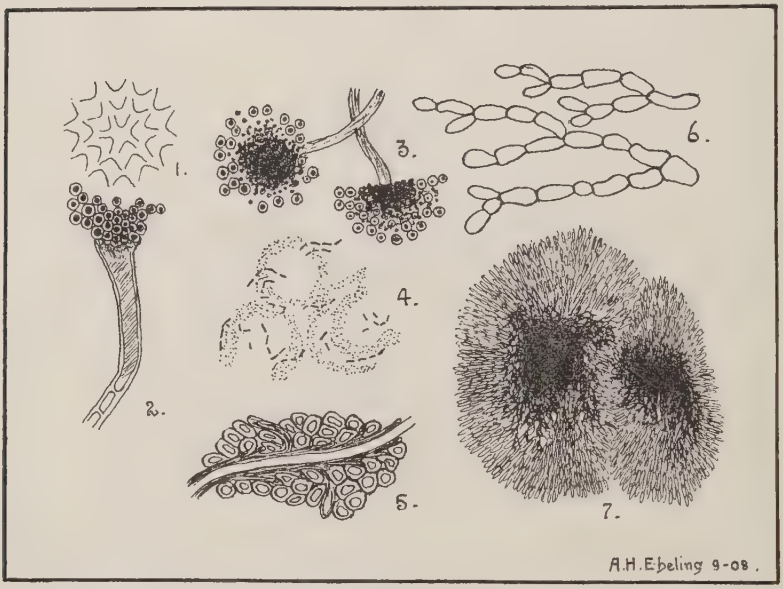

FIG. 42.—Tropical fungi. 1, concentric rings of Aspergillus concentricus; 2, sporangium of A. concentricus; 3, Aspergillus pictor; 4, Microsporoides minutissimus; 5, Trichosporum giganteum; 6, black granules of Madurella mycetomi; 7, yellow grains of Discomyces maduræ.

Achorion schoenleini is the cause of favus. The cultures are rather wrinkled. It is characterized by the scutulum or favus cup. This is a sulphur-yellow pea-sized cup with a central lusterless hair. Affected hairs may not show a cup. Favus is not so contagious as ringworm. It chiefly affects the hairy scalp, but may also invade the nails and even the body.

Microscopical examination shows great irregularity of spores and mycelium, the latter being irregularly disposed and of varying thickness and length and wavy

instead of straight as in Trichophyton. There is also the greatest irregularity in the refractile favus spores—they are gnarled and bizarre shaped, in contrast to the regular ovals or spheres of the ringworm fungus. Cultures show ridges or convolutions.

In the suborder Carpoascus we have to consider the family Perisporiaceæ. In this family the asci are completely inclosed by the investing membrane, the perithecium. When this rots the spores are set free. There are two genera of interest, Penicillium and Aspergillus.

In Penicillium we have vertical branches with strings of conidia. In Aspergillus these conidia arise from a globular termination of the hypha.

Penicillium.—While Penicillium does at times form perithecia, yet they characteristically show chains of spores. The common P. glaucum resembles a hand with terminal beads, more than the hair pencil, from which the name is derived.

P. crustaceum.—Is the common blue-green mould. It has been deemed pathogenic in cases of chronic catarrh of the eustachian tube and in gastric hyperacidity.

Aspergillus.—These have sterigmata carrying chains of spores, these sterigmata being little processes projecting out from the knob-like termination of the aërial hypha (columella). Of the pathogenic Aspergilli we have:

1. A. fumigatus.—This has been considered as the cause of pellagra. A pulmonary mycosis resembling phthisis may be due to this species.
2. A. repens.—This has been found in the auditory canal and may produce a false membrane.
3. A. flavus.—This has been found in the discharges of chronic ear diseases.
4. A. nidulans has been reported as one of the causes of mycetoma showing white granules. It has also been considered a cause of aural mycosis.
5. A. concentricus.—This is the cause of an important tropical ringworm, tinea imbricata. The scales are dry, like pieces of tissue-paper. There are generally about four rings which do not heal in the center. General appearance is that of watered silk. There are no inflammatory lesions. Common in Malay peninsula. Also found in some parts of the Philippines and in China. Some authorities consider the fungus to be a Trichophyton.
6. A. pictor.—This is the cause of a skin affection of Central America. In the affection colored spots appear on the skin, chiefly on face, forearms, and chest. The disease is attended with a mangy odor. Spots are of various colors; if the superficial epithelium is affected we have a dark violet color. Deeper involvement gives red spots.

Hyphomycetes.—In this order are grouped certain genera which cannot properly be assigned to any of the other orders. They are also designated Fungi Imperfecti, for the reason that the fruiting bodies characteristic of the other orders have not been satisfactorily observed.

Discomyces bovis.—This is the well-known ray fungus, the cause of actinomycosis. In man it is at times found in chronic suppurative conditions attended with much granulation tissue. Such pus may show small yellow-gray granules about the size of a pin's head. When spread out between two slides the central portion

shows a network of mycelium with bulbous thread-like rays going to the periphery. The "clubs" at the periphery are degenerate structures and do not stain by Gram. The central mycelium is Gram positive. This mould is essentially an anaerobe and should be cultivated in a deep glucose agar stab. It may also be cultivated in bouillon. In this it grows at bottom. Growth is dry and chalky. In diagnosis look for the little granules. Curetting of the sinuses may give the "ray fungus" when they are not found free in the pus.

Discomyces maduræ.—This is a ray fungus found in the yellow "fish-roe" granules of madura foot. It is strictly aerobic in cultures, thus differing from actinomycosis. For diagnosis proceed as for D. bovis.

Madurella mycetomi.—This is the cause of the black "gunpowder" granules of madura foot. It is a mycelial mass with rather oval shaped swollen segments. It is at times cultivable on potato and agar as felted masses of gray growth, which later become almost black.

Malassezia furfur.—This is the fungus of tinea versicolor. It is common both in temperate and in tropical climates. It is characterized by dirty yellow spots about covered parts of the body. Scrapings show a profusion of mycelial threads and interspersed spores. It is very difficult to cultivate. The organism usually termed the bottle bacillus is really a fungus having the characteristics of the genus Malassezia. It is thought to be the cause of pityriasis of the scalp.

Microsporoides minutissimus.—This is generally considered as the cause of Erythrasma or dhobie itch, a very common intertrigo of the tropics. It is characterized by its narrow mycelium and small spores. Various fungi are found in this affection. Castellani considers the chief cause of dhobie itch to be a trichophyton, T. cruris.

Clinically this affection shows festooned areas of a bright red color which tend to clear up in the center becoming fawn color. As a result of the intolerable itching and scratching the affection tends to spread from its favorite sites—the inner surfaces of the thighs and the scrotum. The spores and mycelium are abundant at the onset but later, one may not find any evidence of the mould. In some of the rapidly spreading cases I have found a symbiosis of fungus and coccus, the bacterial elements lying packed in aggregations scattered through the mycelial ground work.

Culturally these cocci were S. pyogenes aureus.

Trichosporum giganteum.—This is the cause of a disease of the hairs, known in Columbia as "Piedra," so called from the small gritty-like masses along the length of the hair. These spores are arranged like mosaics about the hair.

Sporotrichum beurmanni.—This fungus has a narrow mycelium (2μ) and branches in all directions. The spores appear as little grape-like clusters of oval spores (3 to 5μ) at the end of a filament. It is readily cultivated, showing as a small white growth about the eighth day.

The fungus of Sporotrichosis develops in tissue by budding, not showing the mycelial growth seen in artificial cultures. Potato makes a good medium and often such cultures show pigmentation.

This mould produces indolent, glistening, subcutaneous tumors which are painless. They may ulcerate and give off a brownish discharge. They resemble tuberculous or syphilitic lesions.

Certain organisms which resemble both moulds and bacteria, having branching

filamentous forms and at the same time having a spore-like method of reproduction, are known under the names Streptothrix or better Nocardia. It is chiefly in various pathological processes of the lungs that they have been observed, but in addition they have been noted in brain, glands, kidney and subcutaneous tissue.

The infections are most likely to be confused with phthisis and glanders. The organisms are easily cultivated and in staining reactions are midway between T. B and Actinomycosis.

Diagnosis of Fungi.

The most expeditious way to examine for fungi is to treat the scales or hairs with a 10% solution of caustic potash or soda. Then crush between two slides; heat moderately over the flame and examine.

Tribondeau's method is to treat the scales with ether, then with alcohol, and finally with water. Next put the sediment (it is convenient to use a centrifuge) in a drop of caustic soda solution. Cover with a cover-glass, and after the preparation has stood about an hour run glycerine under the cover-glass.

A very satisfactory method is to scrape the scales with a small scalpel, and smear out the material so obtained in a loopful of white of egg or blood-serum on a glass slide. By scraping vigorously the serum may be obtained from the patient. After the smear has dried, treat it with alcohol and ether to get rid of the fat. It may then be stained with Wright's stain or by Gram's method. The ordinary Gram method may be used or the decolorizing may be done with aniline oil, observing the decolorization under the low power of the microscope.

Yeasts are best examined in hanging drop on the plain slide with vaselined cell, as given under Blood.

An excellent way to examine moulds is to seize some of the projecting sporangia from the surface of a plate with forceps and mount in liquid petrolatum. I have found that moulds in scales from skin or infecting various mites or insects will show a growth in this medium when mounted on a slide and covered with a cover-glass.

Cultivation of Fungi.

Moulds grow well on media with an acid reaction, so that by adjusting the reaction to +2% or even higher, we permit of the growth of the fungi, but inhibit bacterial development.

Glycerine agar, bread paste, or potato media are all suitable, but the best medium is that of Sabouraud:

Maltose,	4 grams.
Peptone,	1 gram.
Agar,	1.5 grams.
Water,	100 c.c.

Make the reaction about +2.

Before inoculating media with moulds, some recommend placing the material in 60% alcohol for one or two hours to kill the bacteria. The moulds withstand such treatment.

In cultivating moulds it is best to use small Erlenmeyer flasks, containing about one-fourth of an inch of media on the bottom, for the development of the colonies. In order to separate the mould we may take the hair or scales on a sterile slide and cut them into small fragments with a sterile knife. Then moisten a platinum loop from the surface of an agar slant, touch a fragment with the loop, and when it adheres transfer it to the agar slant. Make four or five inoculations on the surface and from suitable growth, after four to seven days, inoculate the medium in the Erlenmeyer flask.

Plauth recommends receiving the mould material between two sterile glass slides. Seal the edge of the slide with wax and place the preparation in a moist chamber for four to seven days. From developing fungus growth inoculate the medium in the Erlenmeyer flask. A Petri dish containing several layers of thoroughly moistened filter-paper in top and bottom makes a satisfactory moist chamber.

CHAPTER XI.

BACTERIOLOGY OF WATER, AIR, MILK, ETC.

Bacteriological Examination of Water.

While in a chemical examination as to the character of a water there are certain relations between the free and albuminoid ammonias, nitrates, nitrites, chlorides, etc., which indicate the probable animal as against vegetable nature of the organic matter present, yet it is a more or less presumptive evidence. In a bacteriological examination of water the finding of the colon bacillus may from a practical standpoint be considered as positive evidence of human fæcal contamination. Theoretically, the possibility of organisms being present corresponding culturally to B. coli and derived from cereals is to be considered. Also the fæces of animals contain an organism which cannot be differentiated from the colon bacillus.

In detecting sewage contamination in water to which varying amounts of sewage had been added, it was found that the bacterial tests were from ten to one hundred times more delicate than the chemical ones.

As showing sewage contamination of water, the presence of the B. coli has been generally accepted as the most satisfactory indication. The English authorities consider sewage streptococci and the spore-bearing B. enteritidis sporogenes as of value as indicators as well as the B. coli—the presence of sewage streptococci indicating very recent sewage contamination and that of the B. enteritidis sporogenes, in the absence of streptococci and colon bacilli, as evidence of sewage contamination at some period more or less remote.

In the United States the colon bacillus alone is considered the indicator of sewage contamination, and all tests, presumptive or positive, are based on the presence of this organism.

It is not the finding of the colon bacillus but rather the question of it's relative abundance that is involved in a water analysis. Thus the finding of one colon bacillus in 50 c.c. of water would not have weight as showing contamination, but the presence rather constantly of the colon bacillus in 1 c.c. or less makes contamination of a water supply probable.

In collecting samples of water for bacteriological examination, the following points should be considered:

1. The bottles, which should have a capacity of from 25 to 100 c.c., should be sterile. Sterilization may be effected by heat or by rinsing with a little sulphuric acid and subsequently washing out thoroughly with the suspected water before collection. The utmost care must be exercised that the fingers do not come in contact with the glass stopper of the neck of the bottle while filling it. If the specimen is to be sent some distance, it should be packed in ice to prevent bacterial development. Frankland states that a count of 1000 became 6000 in six hours and 48,000 in forty-eight hours. In water packed in ice for a considerable time, however, the bacterial count may diminish.

2. If collecting from city water supplies, secure the sample direct from the mains and let the water run from the tap a few minutes before collection. If the water be taken from a pond, stream, or cistern, be sure that the specimen comes from at least 10 inches below the surface. As sedimentation is the most important method in self-purification of rivers and ponds, it will be understood that any stirring up of the mud on the bottom will enormously increase a bacterial count.

Quantitative Bacteriological Examination.

1. Deliver definite quantities of the water to be examined into tubes of liquefied gelatin or agar and plate out the same in a series of Petri dishes.

A more practical method is to deliver the water from the graduated pipette into the empty sterile dish. The water should be deposited in the center of the plate and the melted gelatin or agar poured directly on the water and then, carefully tilting to and fro, mix the water and the media. One set of plates should be of gelatin and incubated at room temperature; a similar set should be of lactose litmus agar and incubated at 38° C. If the water is highly contaminated, it is necessary to dilute it; thus, with river water, which may contain from 2000 to 10,000 bacteria per c.c., a dilution of 1 to 100 would be desirable.

Ordinarily it will be sufficient to deliver from a sterile graduated pipette 0.2, 0.3, and 0.5 c.c. of the water in each of two sets of plates: one set for gelatin, the other for agar.

When gelatin is not at hand or convenient to work with, the gelatin plates may be replaced by others of lactose litmus agar for incubation at room temperature. After twenty-four hours at 38° C. or forty-eight hours at 20° C., the count should be made.

Example.—Forty colonies were counted on the gelatin plate containing 0.2 c.c. (1/5) of the water. The number of organisms would be 200 per c.c. Ten colonies were counted on the agar plate containing 0.2 c.c. and incubated at 38° C. Number of bacteria developing at body temperature equals 50 per c.c.

There is no strict standard as to the number of bacteria a water should contain per c.c. Koch's standard of 100 colonies per c.c. is generally given. It is by the qualitative rather than the quantitative analysis that one should judge a water.

If there should be very many colonies on a plate, the surface can be marked off into segments with a blue pencil. If very numerous, cut out of a piece of paper a space equal to 1 square centimeter. By counting the number of colonies inclosed

in this space at different parts of the plate, we can strike an average for each space of 1 square centimeter. To find the number of such spaces contained in the plate, multiply the square of the radius of the plate by 3.1416. Then multiply this number by the average per square centimeter, and we have the total number of colonies on the plate. This is the principle of the Jeffers disc.

The relative proportion between the bacterial count at 20° C. and that at 38° C. is of great importance from a qualitative standpoint, as will be seen later.

2. Deliver into a series of Durham fermentation tubes containing glucose bouillon and into another series containing lactose bouillon varying definite amounts of the water to be examined. In tubes showing the presence of gas in both glucose and lactose bouillon the evidence is presumptive that the colon bacillus is present. For the positive demonstration plates must be made from such tubes as show gas.

It is sufficient to deliver from graduated pipettes in each series quantities of water varying in amount from 0.1 c.c. to 10 c.c. In our laboratory we inoculate with 0.1 c.c., 0.2 c.c., 0.5 c.c., 1 c.c. and 10 c.c. of the suspected water. If the 0.1 c.c. tubes show gas, we have reason to assume that the water contained at least 10 colon bacilli per c.c. If only the 10 c.c. tubes showed gas—those with less amounts not having gas—we would be in a position to state that the water contained the colon bacillus in quantities of 10 c.c., but not in quantities of 1 c.c. or less. Many authorities regard water as suspicious only when the colon bacillus is present in quantities of 10 c.c. or less; waters of good quality frequently showing the presence of the colon bacillus in quantities of 100 to 500 c.c.

It is generally accepted that if a water shows the presence of the colon bacillus in quantities of 1 c.c. or less, it should be regarded as suspicious.

At the present time the medium that gives the least source of error in carrying out the quantitative presumptive tests is the lactose bile. It is made by adding 1% of lactose and 1% of peptone to ox bile, and fermentation tubes of the media showing gas may be considered as very probably containing the colon bacillus. The percentage of error with this method is reported to be only 11%, while with glucose fermentation tubes the error is more than 50%. Gas formation is usually shown in forty-eight hours, but it is advisable to continue the incubation for seventy-two hours. These presumptive tests are chiefly of value in highly contaminated waters. Even with this method plates should be made.

3. As the colon and sewage streptococci ferment lactose with the production of acid and hence produce pink colonies on lactose litmus agar, much information can be obtained from the proportion existing between the number of pink colonies and those not having such a color. Waters of fair degree of purity rarely give any pink colonies.

Qualitative Bacteriological Examination.

General Considerations.—In some countries the proportion of liquefying to nonliquefying colonies on gelatin plates is considered of importance. Certain sewage organisms belonging to the proteus and

cloaca groups liquefy gelatin; consequently, if the proportion of liquefying to nonliquefying be greater than as 1 to 10, the water is considered suspicious. The test is not considered by American authorities as of any particular value.

The American Public Health Association recognizes the importance of the information obtained from a comparison of the number of organisms developing at 38° C. and those developing at 20° C. Bacteria whose normal habitat is the intestinal canal naturally develop well at body temperature, while normal water bacteria prefer the average temperature of the water in rivers and lakes. Consequently when the number of organisms developing at 38° C. at all approximates the number developing at 20° C., there is a strong suspicion that sewage organisms may be present. Normal waters give proportions of 1 to 25 or 1 to 50, while in sewage contaminated waters the proportion may be as 1 to 4 or less.

In addition, the appearance of pink colonies on the lactose litmus agar is of great assistance in judging of a water. Both sewage streptococci and the colon bacillus give pink colonies—those of the streptococci are smaller and more vermilion in color. Microscopic examination will differentiate the cocci from the bacilli. It is well to bear in mind that the pink colonies after twenty-four hours may turn blue in forty-eight hours from the development of ammonia and amines. Consequently the lactose litmus agar plates should be studied after twenty-four hours.

A good water supply will rarely show a pink colony, while in a sewage-contaminated one the pink colonies will probably predominate.

The diagnostic characteristics considered important by the American authorities in reporting the colon bacillus (Recently designated excretal colon bacillus) are:

1. Typical morphology, nonsporing bacillus, relatively small and often quite thick.

2. Motility in young broth cultures. (This is at times unsatisfactory, as some strains of the colon bacillus do not show it even in young bouillon cultures).

3. Gas formula in dextrose broth. Of about 50% of gas produced, one-third should be absorbed by a 2% solution of sodium hydrate (CO_2). The remaining gas is hydrogen. (Later views indicate that the gas formula is exceedingly variable and should not be depended upon. To carry out this test one fills the bulb of a fermentation tube with the caustic soda solution, holding the thumb over the opening or with a rubber stopper, the bouillon culture and the soda solution are mixed by tilting the fermentation tube to and fro. The total amount of gas is first re-

corded and then that remaining after the CO_2 has been absorbed is reported as hydrogen.)

4. Nonliquefaction of gelatin.
5. Fermentation of lactose with gas production.
6. Indol production.
7. Reduction of nitrates to nitrites.

To these may be added the acidifying and coagulation of litmus milk without subsequent digestion of the casein. The production of gas and fluorescence in glucose neutral red bouillon is also a very constant function of the colon bacillus. B. coli aerogenes is similar to B. coli with the exception of nonmotility and production of gas in saccharose media. B. coli anaerogenes is also similar to B. coli but does not produce gas in glucose and lactose.

NOTE.—The reduction of neutral red with a greenish-yellow fluorescence is very striking and has been suggested as a test for the colon bacillus. Many other organisms, especially those of the hog cholera group, have this power. It is convenient, however, to color glucose bouillon with about 1% of a 1/2% solution of neutral red.

On the plates made for the detection of colon bacillus may be found certain organisms which have origin in fecal contamination. The more important of these are those of the paratyphoid, cloaca and proteus groups. In addition, the B. fecalis alkaligines has not rarely been isolated. Among natural water bacteria there may be present either the liquefying or the nonliquefying B. fluorescens. These colonies have a yellowish-green fluorescence.

Certain chromogenic cocci and bacilli are found in uncontaminated waters as B. indicus or B. violaceus. From surface washings we obtain certain soil bacteria as B. mycoides, B. subtilis, B. megatherium. One of the higher bacteria which shows long threads, Cladothrix dichotoma, is common, and is characterized by a brown halo around it's gelatin plate colony.

Isolation of the Typhoid Bacillus from Water.

This is probably the most discouraging procedure which can be taken up in a laboratory. Only the most recent reports of such isolation from water supplies, which have been verified by immunity reactions, can be accepted and of these the number of instances is exceedingly small. Owing to the long period of incubation, the typhoid organisms may have died out before the outbreak of an epidemic suggests the examination of the water supply.

There have been various methods proposed for the detection of the B. typhosus in water. A method which would offer about as reasonable a chance of success as any other would be to pass 2 or 3 liters of the water through a Berkefeld filter; then

to take up in a small quantity of water all the bacteria held back by the filter. Then plate out on lactose litmus agar and examine colonies which do not show any pink coloration. The dysentery bacillus has about the same cultural characteristics as the typhoid one, so that it is important to note motility. If from such a colony you obtain an organism giving the cultural characteristics of B. typhosus, carry out agglutination and preferably bacteriolytic tests as well. Some strains of typhoid, especially when recently isolated from the body, do not show agglutination.

The Conradi Drigalski, the malachite-green, and various caffeine containing plating media have been highly recommended.

Isolation of the Cholera Spirillum from Water.

The method proposed by Koch in 1893 does not seem to have been improved upon by later investigators. To 100 c.c. of the suspected water add 1% of peptone and 1% of salt. Incubate at 38° C., and at intervals of eight, twelve, and eighteen hours examine microscopically loopfuls taken from the surface of the liquid in the flask. So soon as comma-shape organisms are observed, plate out on agar. The colonies showing morphologically characteristic organisms should be tested as to agglutination and bacteriolysis. Inasmuch as the true cholera spirillum shows a marked cholera-red reaction it is well to inoculate a tube of peptone solution from such a colony and add a drop of concentrated sulphuric acid after incubating for eighteen hours. The rose-pink coloration is given by the cholera spirillum with the acid alone—the nitroso factor in the reaction being produced by the organism.

Bacteriological Examination of Milk.

A bacterial milk count is of comparatively little value as showing whether a milk is dangerous or not. As a matter of fact, a milk which contains several million of bacteria per c.c. might be less dangerous than one containing only a few thousand, especially if in the latter there were numerous liquefiers and gas producers present. There is, however, one point of importance in connection with the quantitative estimation of bacteria in milk, and that is the fact that in order to keep the development of the bacteria within the limits of 10,000 to 50,000 per c.c., it is necessary that the requirements of cleanliness in milking and the rapid cooling of the milk after obtaining it and the keeping of the temperature below 50° C. be rigidly observed. If a milk has a high count it shows some error in the handling of the milk. Anderson has found that top milk contains from ten to five hundred times as many bacteria as bottom milk. Centrifugally raised cream contains more bacteria than that forming by gravity. In making a quantitative bacteriological examination, the principle is the same as with water.

Make a known dilution of the milk with sterile water; add definite quantities of this diluted milk to tubes of melted agar or gelatin, and pour into plates. The diluted milk may also be delivered in the center of the plate and the melted agar or gelatin poured directly on it, mixing thoroughly. Always shake the bottle well before taking sample.

Example.—Added 1 c.c. of milk to 199 c.c. of sterile water in a large flask (500 to 1000 c.c.) After shaking thoroughly, take 1 c.c. of this 1 : 200 dilution and add it to 99 c.c. of sterile water. Shaking thoroughly, we have a dilution of 1:20,000. Of this we added 0.5 c.c. to a tube of gelatin or agar. After incubation the plate showed 75 colonies. Therefore the milk continued in each c.c. $75 \times 2 \times 20{,}000$ (dilution) $= 3{,}000{,}000$—the number of bacteria in each c.c. of milk.

Lactose litmus gelatin or agar is to be preferred in milk-work, as the normal lactic acid bacteria produce reddish colonies which are very striking. A standard easily attained for high-grade, certified milk would be 5000 to 10,000 per c.c.

In the qualitative examination of milk, many dairies employ the fermentation tube, any organism producing gas being considered undesirable. Again liquefying organisms, as shown by the presence of such bacteria in the gelatin plates, is evidence of probable contamination by fæcal bacteria. A question which seems difficult to decide is as to the general nature of the so-called normal lactic acid bacteria of milk. Some describe them as very short, broad bacilli with very small colonies, fermenting lactose with the formation of lactic acid. Others consider that the streptococci are the organisms which are concerned with the normal fermentative changes. In examining specimens of milk considered the best on the market, I have repeatedly found the small red colonies on lactose litmus agar to be in chains of either Gram positive streptococci or streptobacilli. Of the acid-forming bacilli in milk we have 1. the B. lactis acidi group. These are oval cells about 0.9 microns by 0.6 microns, often in chains. They are Gram positive and nonmotile. They may be the same as Streptococcus lacticus of Kruse. They curdle milk with a homogeneous clot—this being due to the fact that they do not produce gas in lactose media. 2. The B. coli aerogenes group. These are gas producers. (See under water.) 3. The B. bulgaricus group. In connection with the organisms present in the tablets used for treating milk to produce lactic acid for the treatment of intestinal disorders, and considered to be normal lactic acid bacteria, I have found both streptococci and bacilli. These have all agreed, however, in not producing gas in either lactose or glucose fermentation tubes.

The organism upon which special stress is laid in these so-called lactic acid producers is the B. bulgaricus. This is a large, nonmotile organism with square ends like anthrax. It often occurs in long chains and does not possess spores. It is Gram positive and often shows metachromatic granules like those of the diphtheria bacillus. Colonies show in forty-eight hours which resemble streptococcus ones, but are more contoured on the surface. It produces a deep vivid pink in litmus milk, while milk streptococci only cause a light pink. It produces a very large amount of acid (3%). Little or no growth on ordinary laboratory media or below 0° C. (Op. temp. 42° C.).

Heinemann states that it occurs normally in human fæces and various fermented milks—also in gastric juice when HCl is absent. To isolate, put milk or fæces into a broth containing 0.5% acetic acid and 2% glucose. Transfer to litmus milk after

twenty-four hours and from such tubes plate out on milk serum agar (coagulate boiling milk with a few drops of acetic acid, filter and add 1% peptone, 2% glucose and 1.5% agar).

As they grow in very acid media the term acidophilous is applied. It was supposed that these bacteria were peculiar to certain fermented milks as matzoon and yogurt. Hastings has shown the group to be present in milk in the United States and considers the source to be the alimentary tract of cows.

Another source of information as to the quality of a milk may be derived from a study of the number of leukocytes or pus cells contained in 1 c.c. of the milk. It must be understood that cellular elements which differ only slightly from true pus cells may be found in the milk of healthy cows and may be found in great numbers. Statements have been made that such cells are neither amœboid nor phagocytic.

The Doane-Buckley method is probably the most accurate. In this you throw down the cellular contents of 10 c.c. of milk in a centrifuge revolving about 1000 times a minute for ten to twenty minutes. Then remove supernatant milk and add 0.5 c.c. of Toisson's solution to the sediment. You thus have the leukocytes of 10 c.c. contained in 0.5 c.c. (Concentrated twenty times.) Make a hæmatocytometer preparation as for blood and find the average munber of cells for each square millimeter. Then multiply this by 10 to get the number of cells in a cubic millimeter. As a cubic millimeter is one thousand times smaller than a cubic centimeter, you multiply the number per cubic millimeter by 1000. Then, as the milk was concentrated twenty times, you divide by 20. (If it were diluted twenty times, you would multiply by 20.)

Example.—Found an average of 50 cells per square millimeter. This would make 500 per cubic millimeter, and 500,000 per c.c.; then 500,000 divided by 20 would give 25,000.

There is no agreement as to a standard for allowable leukocytes. Even in apparently healthy animals they may exceed 100,000 per c.c. Doane has suggested 500,000 per c.c. as a preferable limit.

The smear methods for determining the number of leukocytes present do not compare in accuracy with the volumetric ones.

To summarize, we may state that the bacterial count is an indicator of the care used in handling the milk while the presence of harmful bacteria (qualitative examination) or numerous pus cells indicates disease in the cow. During 1912 severe epidemics of sore throat due to a streptococcus, S. epidemicus, were traced to milk of cows having probably suffered from mastitis. In Baltimore the milk had been pasteurized by the flash method which indicates the unreliability of this process.

Pasteurization of Milk.—The objections to this method of preserving milk have been (1) that the lactic acid bacteria which have been by some credited with

antagonism to harmful bacteria, would be destroyed by pasteurization, (2) the more rapid development of bacteria in milk that has been pasteurized (3) interference with nutritive qualities and (4) pasteurized milk does not show its deterioration as does unpasteurized milk, thus failing to give a clue as to the age of the milk.

The United States Bureau of Animal Industry in studying this important phase of the milk question has grouped the milk bacteria into three classes (a) acid-forming, (b) putrefactive (liquefying) and (c) inert bacteria. In their investigations it was found that many acid-forming bacteria withstood temperature as high as 168° F., so that pasteurized milk was soured just as is raw milk, but more slowly. They found that pasteurized milk showed fewer putrefactive bacteria than raw milk, so that even should it be a fact that injurious toxins were produced by spore-bearing putrefactive organisms the development of such organisms would be even less in pasteurized milk.

The statement so often advanced that bacteria develop more rapidly in pasteurized milk than in raw milk was proved fallacious.

It was recommended that holding the milk for thirty minutes at 145° F. was a far better method of pasteurizing than quickly bringing the milk to a temperature of 185° F. (flash method). All admit the great value of the killing of important pathogens (typhoid, cholera, streptococci, etc.).

Bacteriological Examination of Air.

In Paris a cubic meter of air was found to contain the following number of organisms:

Suburbs.—Winter,	145 moulds,	170 bacteria.
Summer,	245 moulds,	345 bacteria.
City Hall.—Winter,	1345 moulds,	4305 bacteria.
Summer,	2500 moulds,	9845 bacteria.

Air of hospitals, especially after sweeping, may contain 50,000 bacteria per cubic meter. There does not seem to be any particular relation between the amount of carbon dioxide in air and the bacterial content.

Petri's Rough Method.—Exposure of a lactose litmus agar plate (capacity 100 sq. cm.) for five minutes will give the number of organisms present in ten liters of air. Multiply by 100 for one cubic meter.

The two groups of organisms usually found in air are 1. bacteria and 2. moulds. Moulds (spores) may be carried by currents of air; bacteria, however, are generally carried about by particles of dust or finely divided liquids (spray). On the lactose litmus agar plate staphylococci and streptococci show as bright red colonies.

Sedgwick-Tucker Sterile Granulated Sugar Method.—Sterilize aerobioscope and introduce granulated sugar on support. Again sterilize (not over 120° C. in dry-air sterilizer). Allow a given quantity of air to pass through; then shake the sugar into wide part of aerobioscope. Now pour in 10 or 15 c.c. of melted gelatin

(40° C.) to dissolve sugar. Roll tubes as for Esmarch roll cultures, and incubate at room temperature. To draw air through the aerobioscope, connect the small end with a piece of rubber tubing which is attached to a tube in the stopper of an aspirating bottle. Having poured a definite quantity of water into the aspirating bottle, allow the water to run out. The same quantity of air will be drawn through the sugar of the aerobioscope as the amount of water passing out of the aspirating bottle. The bacteria and moulds are caught by the sugar.

Example.—Passed ten liters of air through the aerobioscope. The bacteria in this quantity of air showed 75 colonies when incubated at 20° C. The unit being one cubic meter or one thousand liters, we have only obtained the bacteria of one hundredth of the unit. Hence multiplying 75 by 100 gives 7500 bacteria as present in one cubic meter of the air examined.

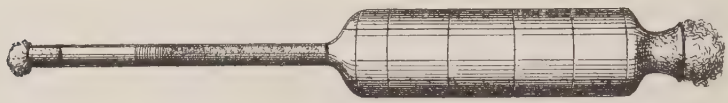

Fig. 43.—Sedgwick-Tucker aerobioscope. (*Williams.*)

In comparing the results with the aerobiscope with those obtained by exposing a plate as in Petri's method for ten instead of five minutes, it was found that the latter was sufficiently in accord to make it a satisfactory approximate quantitative method. The simplicity and ease of access to the colonies developing on it make it preferable when the air of operating-rooms or hospital wards is to be examined.

Of the fungi ordinarily obtained in examinations of the air the blue-green mould and the red yeast are the most common. B. subtilis and sarcina types of cocci are the most common bacterial colonies found upon exposed plates. Sewer air is as a rule free from bacteria, due probably to the fact that bacteria tend to adhere to moist surfaces. The importance of Flügge's droplet method of contamination of the air of a room is brought out in the discussion of infection with pneumonic plague. This is an important method in the transmission of tuberculosis.

CHAPTER XII.

PRACTICAL METHODS IN IMMUNITY.

That which prevents the gaining of a foothold by disease organisms in the animal body or which neutralizes their harmful products or destroys the parasites is termed immunity. In the main, the question of immunity hinges on the powers of resistance of the human body and the aggressiveness or virulence of the invading organism. It must always be kept in mind that immunity is only relative; thus the fowl, which is practically immune to tetanus, may be made to succumb by reducing its resistance by refrigeration or by increasing the amount of poison introduced. The insusceptibility which the fowl has to tetanus or which man has to many diseases of animals is best termed inherent immunity, and is at present only a subject of theoretical interest. When immunity to a given disease is obtained as a result of an attack of the disease in question or by laboratory methods of inoculation, this is termed properly an acquired immunity, and in the former case is a naturally acquired immunity or "natural immunity" and in the second is an artificially acquired immunity or "artificial immunity."

Immunity may be divided into that which is inherent and that which is acquired. Inherent immunity is such as is observed in the resistance of Algerian sheep to anthrax (ordinary sheep are very susceptible) or the fowl to tetanus and is of interest theoretically rather than practically. Acquired immunity may be brought about naturally as by an attack of a disease or artificially by laboratory measures.

As a result of an attack of a disease or in response to the stimulus of the injection of the organisms or its products, we have developed in the man so injected certain specific antagonistic properties to that organism, which are usually demonstrable in the blood-serum or other body fluids, and to which we apply the terms agglutinating power, opsonic power, or bacteriolytic power. The term antibody is also applied. All three powers may be present together in equal or in varying degree or one or more may be absent. By agglutinating power we mean that which causes evenly distributed organisms to come together and form clumps. By opsonic power we mean that which so alters the

resistance of bacteria that the phagocytes ingest them. By bacteriolytic power we mean that which brings about disintegration or lysis of the specific organism. The bacterium which causes the disease or which is used in inoculation for the production of immunity is termed the specific organism.

Of the different kinds of immunity only artificial immunity will be considered. This may be obtained in two ways: By injecting the bacteria or their products into man or animals and as the result of the activity of the cells of the animal invaded, antibodies are formed which neutralize the toxins (antitoxins) or bring about lysis of the specific bacteria (bacteriolysins). These antibodies which are supposed to be thrown off (free receptors) from those body cells which have suitable fixation powers for the invading toxin molecule or bacterium may remain potential for months or years and so confer a more or less enduring immunity.

These fixation points are known as cell receptors and are intended for the assimilation of various foodstuffs by the cell. If destroyed by the toxin or bacterium they are reproduced in great excess by nature.

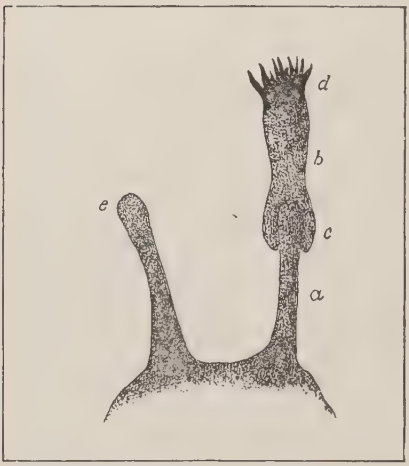

FIG. 44.—Receptors of the first order uniting with toxin. (*Journal of the American Medical Association*, 1905, p. 955.) *a*, Cell receptor; *b*, toxin molecule; *c*, haptophore of the toxin molecule; *d*, toxophore of the toxin molecule; *e*, haptophore of the cell receptor.

Not only may bacteria act in this way but foreign cells, such as red cells or various parenchymatous cells, when injected, give rise to antagonistic substances which act as factors in their destruction—hæmolysins for red cells, cytolysins for different parenchymatous cells. The substance which is injected and in reaction to which antibodies are produced is called an antigen. This is termed "active immunity."

When we take the serum of a man or animal immunized actively and inject it with its contained antibodies into a second animal or man, we confer an immunity on the second animal; but as his cells take no active part in the production of the immunity, but are only passive, we term this immunity "passive immunity." If this serum which is introduced in passive immunity only neutralizes the toxic products of the infecting bacteria, we term it antitoxic passive immunity and designate the immune serum as antitoxic serum. If it destroys the organism, we call it anti-

microbic serum, and the immunity, antimicrobic passive immunity. Some immune sera are both antitoxic and antimicrobic.

It is well to remember that some organisms produce a toxin which is given off while the bacterium is alive; and in other instances the toxin is intracellular and is only given off when the bacterium disintegrates; consequently, an antimicrobic serum may cause the liberation of toxin. Diphtheria, tetanus, or botulism antisera are instances of antitoxic sera, while practically all others are antimicrobic. There is but one factor to consider in an antitoxic serum and that is the protoplasmic particles which are thrown off from the cell in response to the injury incident to the attack upon the cell by the toxin particles. This free particle in the circulation represents the entire mechanism of antitoxic-immunity. It is capable of uniting with the toxin molecule and neutralizing its toxic power, or rather so binding its combining end (haptophore group) that it is incapable of attaching itself to a cell, so that the poisonous end of the toxin (toxophore group) cannot have access to the cell.

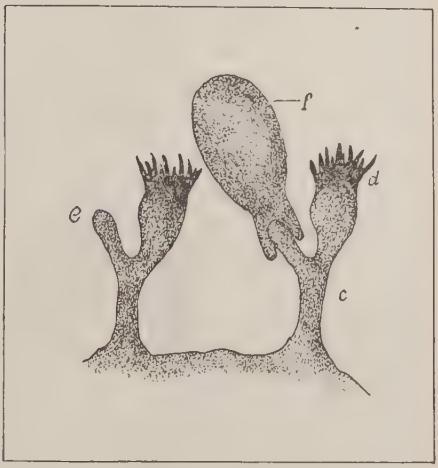

FIG. 45.—Receptors of the second order and of some substance uniting with one of them. *Journal of the American Medical Association*, 1905, p. 1113.) *c*, Cell receptor of the second order; *d*, toxophore or zymophore group of the receptor; *e*, haptophore of the receptor; *f*, food substance or product of bacterial disintegration uniting with the haptophore of the cell receptor.

The term toxin, strictly speaking, is applicable only to such bacterial poisons as (1) require a period of incubation before being capable of manifesting toxic symptoms and (2) can produce antitoxins.

(For further discussion of toxins and antitoxins see under diphtheria, tetanus, botulism, and pyocyaneus infections.) In antimicrobic sera we have two factors to consider, the first is a protoplasmic particle quite similar to the antitoxin molecule, but which in itself has no power of injuring its specific bacterium. This particle is generally referred to as the amboceptor or immune body. It is the specific product of the activity of a specific bacterium or foreign cell against the body cells attacked. It withstands a temperature above 56° C. and of itself is incapable of injuring the bacterium in response to whose attack it was produced. The second factor in the bacteriolysis of the specific bacterium, or the hæmolysis of the specific foreign cell, is something normally present in the serum of every animal, and which is capable of disintegrating a foreign cell or bacterium, provided it can have access to the cell or bacterium through an intermediary amboceptor (hence the amboceptor is some-

times called an intermediary body). This something is called the "complement." It is by some called "alexine," by others cytase (Metchnikoff). The complement cannot act upon and destroy an invading bacterium or cell unless the amboceptor is present to make the necessary connection. The complement is destroyed by a temperature of 56° C., so that, if we heat the serum from an immune animal to 56° C., the complement it naturally contains is destroyed, and the amboceptor it contains which is not injured by such a temperature, is incapable of destroying bacteria or cells, unless we replace the complement which has been destroyed by fresh complement. This is done experimentally by adding the serum of a nonimmunized animal which contains the complement, but no specific immune body (amboceptor) to the heated serum. This is termed "activating," and a serum so treated is said to be "activated." When an immune serum has been heated to 56° C., it is said to have been "inactivated."

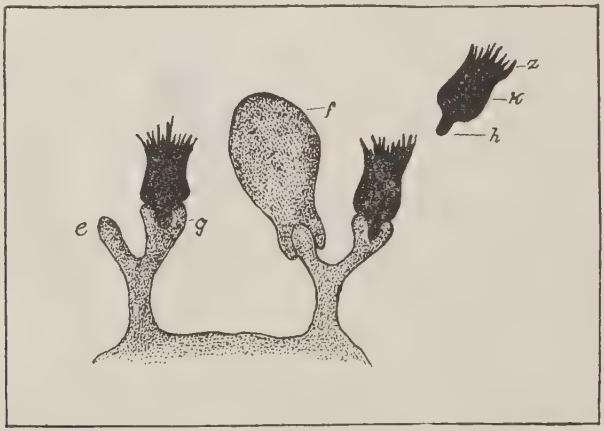

FIG. 46.—Receptor of third order, and of some substance uniting with one of them. (*Journal of the American Medical Association*, 1905, p. 1369.) *c*, Cell receptor of the third order—an amboceptor; *e*, one of the haptophores of the amboceptor, with which some food substance or product of bacterial disintegration (*f*) may unite; *g*, the other haptophore of the amboceptor with which complement may unite; *k*, complement; *h*, the haptophore; *z*, the zymotoxic group of complements.

Antimicrobic sera are not as efficient in treatment as antitoxic ones. It might be that if we could use homologous sera for treating man instead of the usual heterologous ones from the horse better results might obtain.

It would appear that a more hopeful outlook will obtain by combining serum therapy with chemo-therapy, thus a combination of antipneumococcic serum with sodium oleate seems capable of producing curative results which neither alone can bring about.

Again, a combination of vaccination (active immunization) with

the injection of the antimicrobic serum (passive immunization) has been thought by some to be of value. When we allow a mixture of bacteria or cells to remain in contact with their specific immune serum which has been inactivated, the amboceptors attach themselves to the bacteria or cells, so that now, upon adding normal serum (complement), these bacteria or cells are so prepared that the complement can disintegrate them. This experiment is termed "sensitizing" and cells so treated are said to be "sensitized."

Methods for Obtaining Immune Sera.

While a convalescent from a disease may be utilized to obtain an antitoxic, agglutinating, opsonic, or bacteriolytic serum against the specific bacterium, yet this is more conveniently obtained from an animal which has been immunized against the bacterium or cell in question. The rabbit is the most convenient animal to employ for the production of immune sera where the object is to have at hand a serum for use in diagnosis.

Where sera are used on an extensive scale, as in the production of curative sera, larger animals are employed. There are two applications of serum diagnosis: 1. Where the bacterium is known and the serum is to be diagnosed. 2. Where the serum is known and the bacterium is to be diagnosed.

The first is employed by testing the agglutinating or bacteriolytic power of the serum taken from a patient upon pure cultures of the organism which is suspected as the cause of the disease. The Widal test (agglutination) is the best instance of this procedure. This method is of practical value in the diagnosis only of typhoid, Malta fever, and para-typhoid. In diseases like cholera and bacillary dysentery, the disease has run its course before agglutinating power becomes apparent in the serum. This method, however, may be used to prove that a convalescent has suffered from a suspected disease. Thus, by testing the agglutinating power of a serum, one or two weeks after recovery from a suspicious case of ptomaine poisoning, we may be able to demonstrate that the case in question was cholera. The second method has wider application, and is the one in which we use the sera of animals which have been immunized with known bacteria. Organisms isolated from urine, fæces, or blood of patients, or those obtained from water or food supplies may be identified by testing the agglutinating, opsonic, or bacteriolytic power of known sera against them. This has a wide range of applicability. The testing of the opsonic power of the sera in man or animals immunized against plague, and possibly cerebrospinal meningitis, seems to give more definite information than do agglutination or bacteriolytic tests. With the majority of other organisms, however, the agglutination test is the one almost always preferred.

Even in a small laboratory there are no particular difficulties in the way of having on hand rabbits immunized against typhoid, paratyphoid, Malta fever, acid-producing and nonacid-producing strains of dysentery, cholera, etc. Just as we inject men with vaccines prepared from various bacteria in opsonic therapy, so we inject animals to produce sera for diagnosis. We may use either a bouillon culture or the growth on agar slants taken up with salt solution as the inoculating material. This is heated for one hour at 60° C. to kill the bacteria. Where we desire to produce a serum which will disintegrate red blood cells (hæmolytic serum), we inject about 5 c.c. of the washed red cells of the animal for which we wish to produce a specific

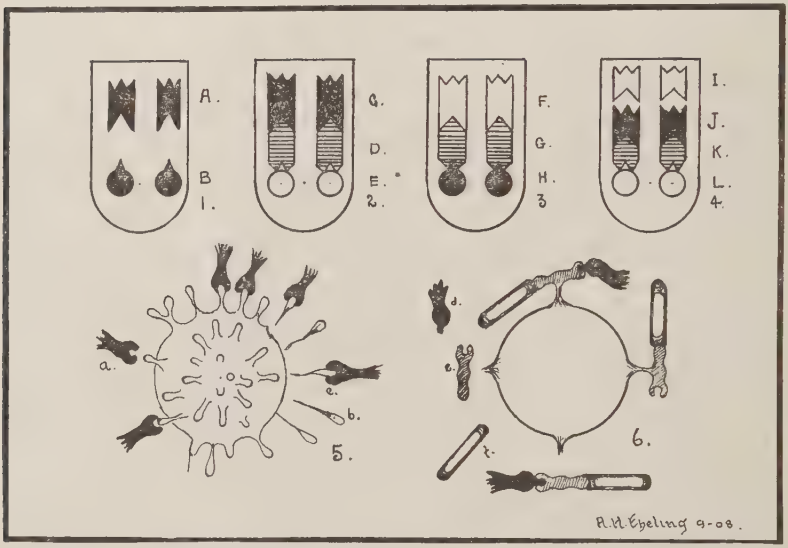

FIG. 47.—1, Red cells + normal serum. No amboceptor. No hæmolysis. A, Complement; B, normal red cell. 2, Red cells + immune serum. Complement and amboceptor. Hæmolysis. C, Complement; D, amboceptor; E, hæmolyzed red cell. 3, Red cells + immune serum heated to 56° C. Inactivated. Complement destroyed. No hæmolysis. F, Destroyed complement; G, amboceptor; H, red cells. 4, Red cells + heated immune serum + fresh serum. (Activated by contained complement.) Hæmolysis. I, Destroyed complement; J, fresh complement; K, amboceptor; L, hæmolysed red cell. 5, Diagram showing antitoxin production. *a*, Toxin molecule; *b*, antitoxin molecule; *c*, neutralization of toxin by antitoxin. 6, Diagram showing bacteriolysin. *d*, Complement; *e*, amboceptor; *f*, bacillus.

serum. For details see method of preparing hæmolytic amboceptor serum under Noguchi's modification of Wassermann test. For preparing a serum for the biological blood test we inject the rabbit with human serum in quantities of about 5 c.c. every fifth day. About one week after the last injection the antiserum obtained from the injected rabbit should be strong enough for one-tenth of a c.c. to produce turbidity when added to 1 c.c. of a 1–1000 dilution of human serum in salt solution. Various controls are necessary when used in medico-legal work.

For obtaining an agglutinating or bacteriolytic serum for bacteria we inject about 1 c.c. of the killed bacterial bouillon culture subcutaneously or into the peritoneal cavity of the rabbit. The easiest way to inject the rabbit is to hold the animal head down and plunge the needle in the median line into the abdominal cavity, forcing in the contents of the syringe. The intestines gravitate downward and by entering the needle below the limits of the bladder we avoid injuring any vital part. It may be more satisfactory to at first inject only about 1/2 c.c., and then if there is very little reaction, as shown by the appetite and spirits of the rabbit, to inject about four days later 1 c.c. About four or five injections at intervals of three to five days will usually produce an immune serum.

Injection of the antigenic material (blood cells, serum or bacterial emulsion) into the marginal ear vein may be employed. With this method, however, I have had several rabbits die in what was considered anaphylactic shock. (For the method of immunizing rabbits to produce a hæmolytic serum see Wassermann test.) Some animals do not seem to be capable of producing antibodies, so that it may be necessary to use one or more rabbits before a satisfactory serum is obtained. The most convenient way of obtaining serum for a test is to cut across one of the marginal veins of the rabbit's ear, and collect the blood in a Wright's U-tube. Centrifugalizing, we have the serum ready for use.

The vein can be made to stand out prominently by applying a compress dipped into very hot water. When a large amount of serum is desired it is better to use a test-tube with two pieces of glass tubing passing through a double perforated rubber stopper. To one of the projecting pieces of glass tubing a stout hypodermic needle is attached through the medium of 8 inches of rubber tubing and to the second piece of glass tubing passing through the stopper of the large test-tube another piece of rubber tubing is attached for suction. To obtain blood from the rabbit find the ensiform cartilage and insert the needle in the notch to the left and gently force it upward. Applying suction with the mouth the blood flows into the test-tube so soon as the needle enters the heart. By placing the tube of blood in the refrigerator the serum separates out from the clot. The removal of 20 to 30 c.c. of blood does not seem to affect the animals in the least and they can be used in this way time and time again. The immune body and agglutinin in serum remain active for weeks when kept in the refrigerator. The complement and opsonin, however, begin to deteriorate at once and have disappeared by the fifth day. Consequently, for opsonic and bacteriolytic and hæmolytic experiments, fresh serum —twelve to twenty-four hours—must be used, or it may be activated.

Agglutination Tests.

There are two methods of testing the aggultinating power of a serum—the microscopical and the macroscopical or sedimentation method.

1. For the microscopical method draw up serum to the mark 0.5 of the white pipette. Then draw up salt solution to the mark 11. This when mixed gives a dilution of 1 to 20. One loopful of the diluted serum and one loopful of a bouillon

culture or salt solution suspension of the organism to be tested gives a dilution of 1 to 40. One loopful of the 1–20 diluted serum and 3 loopfuls of the bacterial suspension give a dilution of 1–80. These two dilutions answer in ordinary diagnostic tests. The red pipette with a 1–100 or 1–200 dilution may be used where dilutions approaching 1–1000 are desired. Having mixed the diluted serum and the bacterial suspension on a cover-glass, we invert it over a vaselined concave slide and examine with a high power, a dry objective (1/6 in.). It is simpler to make a ring of vaseline to fit the cover-glass and make the mixture of diluted serum and culture in the center of this ring or square. Then apply the cover-glass, press it down on the vaseline ring and examine as with the ordinary hanging drop. In making dilutions it is preferable to use salt solution, as the phenomenon of agglutination requires the presence of salts. Ordinarily, thirty minutes is a sufficient time to wait before reporting the absence of agglutination. Agglutination is more rapid at body temperature than at room temperature. In reporting agglutination, always give time and dilution. It is absolutely necessary that a control preparation be prepared in every instance; that is, one with the bacterial culture alone or with a normal serum of the same dilution as the lowest used. Some normal sera will agglutinate in 1 to 10 dilution, and group agglutinations (as paratyphoid with typhoid serum) may occur in 1 to 40 or possibly higher. It is very unusual for sera to agglutinate any other bacteria than the specific one in dilutions as high as 1–80.

2. For the macroscopical or sedimentation test, take a series of small test-tubes (3/8×3 in.) and deposit 1 c.c. of salt solution in each of the series. Now, having taken an empty test-tube, drop 4 drops of serum in it and then add 12 drops of salt solution. This approximately gives 1 c.c. of a 1–4 dilution of the serum. With a rubber-bulb capillary pipette, which has been graduated to hold 16 drops or 1 c.c., draw up the contents of the tube containing the 1 to 4 serum and add it to the next tube containing 1 c.c. of salt solution. This gives a dilution of 1 to 8. Now mix thoroughly by drawing up and forcing out with the bulb pipette, and then withdraw 1 c.c. and add to the next tube containing 1 c.c. of salt solution. This gives a dilution of 1 to 16. Having mixed as before, again withdraw 1 c.c. of the mixture and add it to the 1 c.c. in the next tube. We now have a dilution of 1 to 32. Again withdrawing 1 c.c. and adding it to the fourth tube containing 1 c.c. of salt solution we have a dilution of 1 to 64. In tube 1 there is 1 c.c. of a dilution of the serum of 1 to 8; in tube 2, there is 1 c.c. of a dilution of 1 to 16; in tube 3 of 1 to 32. Tube 4 contains 2 c.c. of 1 to 64. Now adding 1 c.c. of a culture of typhoid or any other organism, we have the dilution of the serum in each tube doubled. Tube 1 now contains a serum in dilution of 1 to 16, acting on the bacteria; tube 2 of a 1 to 32; tube 3 of a 1 to 64. Now place these tubes in the incubator and, after two to five hours or overnight, we examine for the clearing up of the supernatant fluid. If the serum in a certain dilution agglutinates, the clumps gravitate to the bottom and the upper part becomes clear. If so desired, these dilutions may be carried on to 1 to several hundred in the same way. It is safer to work with dead cultures instead of living ones. To prepare, take a twenty-four-hour agar slant culture of typhoid or paratyphoid and emulsify in salt solution (about 6 c.c. to a slant).

By adding 0.1 of 1% of formalin to the typhoid emulsion and placing in the ice-box the cultures will be found sterile in about three days. The emulsion should be shaken twice daily while undergoing sterilization in the ice-box. Such cultures

are not easily contaminated and appear to retain their agglutinable qualities for several months. The macroscopic methods are preferable with such dead cultures.

A very convenient method in general use in Germany is the following: Make dilutions of serum in ordinary test-tubes (3/4×6 in.) as described for the samll test-tubes. Then take a loopful (2 mg.) of culture from an eighteen to twenty-four-hour-old agar culture and emulsify it thoroughly in the dilution in the first test-tube—repeat the process in the second tube and so on. This procedure is much safer than when live cultures are added with a pipette. Again, the dilution is unchanged by this addition whereas it is doubled when an equal volume of culture is added to the diluted serum. A control should always be made in normal salt solution. After incubating, observe flocculent precipitates (agglutination) by tilting the fluid in the tubes to form a thin layer and to obtain the most advantageous light and look for a fine curdy precipitate (aggutination) or a uniformly turbid emulsion (negative reaction).

The method of using a slide with two vaselined rings, one containing an emulsion in the specific serum and the other in salt solution is of great practical value. This method is described under cholera.

Pfaundler under the designation of a thread reaction showed that organisms tended to grow in thread forms in a culture medium containing the homologous serum. Mandelbaum has suggested this as a means of diagnosing typhoid. Take ordinary bouillon containing 1% of sodium citrate. Inoculate it with a culture of typhoid. Now with a bulb capillary pipette take up one part (as marked by a wax pencil) of the patient's blood and fifteen times as much of the citrated bouillon just inoculated with typhoid. Mix the blood and citrated bouillon on a sterile slide or in a test-tube and after drawing up into the lower part of the expansion of the capillary pipette, seal off the capillary end. Now place the sealed-off pipette upright in an incubator and after four or five hours take out from the expanded end a loopful of the clear supernatant fluid (the blood cells settle to the bottom) and if the typhoid bacilli are in chains instead of being single and motile it shows a positive reaction.

Deviation of the Complement.

It has been found that if there is not sufficient immune body in a mixture of normal serum, containing abundant complement, and bacterial emulsion, only a portion of the bacteria will be destroyed. Increasing the amount of immune body with a constant quantity of normal serum, we reach a point where all the bacteria are destroyed. Now, if we continue to increase beyond this point the addition of immune

serum, the destruction of the bacteria ceases, and the cultures will again contain myriads of living bacteria.

To carry out the test, make a series of tubes containing mixtures of bacteria with the same quantity in each of normal serum. Thus, each tube contains 1/2 c.c. of bacterial emulsion and 1/2 c.c. of 1–10 normal serum. Now inactivate a tube of 1-100 immune serum and to each of the tubes of normal serum and bacterial emulsion add increasing drops of the inactivated 1–100 immune serum. Thus, 1 drop to No. 1 tube, 2 drops to No. 2 tube, and so on. After incubating for two hours, we take a pipette and plate out a fraction of a drop in an agar plate. The limit at which bacteriolysis is complete is shown by there being an absence of colonies.

Beyond or below that point colonies are more or less abundant. The explanation of this phenomenon of deviation or deflection of the complement is that where we have an excess of amboceptors for available receptors on the bacterial cells, only a portion of the amboceptors can attach themselves to their specific bacteria. The free amboceptors, not being able to form a union with the bacterial cell receptors (for which they have a greater affinity), combine with the complement present. Unless the complement be in excess, there will be no free complement left to join onto the amboceptors attached to the bacterial cells, and consequently bacteriolysis does not take place and the plate cultures show an abundance of colonies.

Fixation or Absorption of the Complement.

One of the controversies in connection with the nature of the complement is that regarding the question of the unity of complements or whether there exist different kinds of complements for different amboceptors (unity and multiplicity of complement). To prove that a single complement will act with varying amboceptors, Bordet and Gengou showed that the same complement would activate both hæmolytic and bacteriolytic immune bodies. If to a mixture of typhoid bacteria and inactivated typhoid immune serum some guinea-pig serum is added and the mixture be allowed to remain at 37° C. for two hours, and then sensitized red cells be added and the mixture again be placed in the incubator for two hours, no hæmolysis will be found to have occurred, because the bacteria have absorbed all the guinea-pig complement through the intervening typhoid amboceptors, and there is no complement left to hæmolyze the red cells through the specific blood-cell amboceptors. If, instead of immune typhoid serum, the serum of a normal person had been used, there would have been no amboceptors to unite the complement to the bacterial cells. The complement would then be at hand to unite with the sensitized red cells subsequently added and bring about their hæmolysis, as shown by the ruby color of the

supernatant fluid. This phenomenon of Bordet and Gengou has been utilized by Wassermann for the diagnosis of diseases where cultures are not applicable. It is in the diagnosis of syphilis that it is best known. It having until recently been impossible to obtain cultures of Treponema pallidum, we use an emulsion of the liver of a syphilitic fœtus, which has been filtered so as to be clear, instead of a culture. The syphilitic liver, as can be observed by staining according to Levaditi's method, is packed with spirochætes.

While Noguchi has recently obtained pure cultures of the organism of syphilis yet the antigen prepared from such cultures was not found as satisfactory by Craig and Nichols as that from the liver of a syphilitic fœtus, cases of syphilis which showed strongly positive tests with ordinary antigen not giving a positive test with the specific antigen.

It has now been found that lecithin or, preferably, emulsions of various normal organs may be substituted as antigen for the syphilitic liver, the antigenic power being due to lipoids. Aqueous extracts contain in addition to lipoids, substances which render the antigen unstable—alcoholic extracts are more stable and contain less anticomplement.

For the immune bodies we take the serum of the patient, or if a case of locomotor ataxia or general paresis, the cerebrospinal fluid.

Emery's Technic for the Wassermann Test.

Owing to technical difficulties with the method of making and employing the antigen and amboceptor features of the original Emery test, I have retained the principle of the test but substituted the reagents prepared in exact accordance with Noguchi's directions.

Briefly stated, the principle of Emery's test consists in the employment of fresh human serum for supplying complement and the primary incubating of the hemolytic system (human red cells and rabbit serum immune to human red cells) at the same time as the incubation of the antigen and serum but in separate tubes. Then in the second period of incubation to add these "sensitized" cells to the serum antigen combination.

In Noguchi's method all reagents are incubated together in the first period with the exception of the amboceptor paper (dried serum of rabbit immune to human red cells), which is not added until the period of incubation for complement binding is completed and the second incubation commenced. Time is saved in the Emery technic, inasmuch as the red cells are already sensitized by the hemo-

lytic amboceptor when added to the tubes, and hemolysis shows itself almost immediately in tubes when the complement has not been absorbed by the antigen through syphilitic antibodies.

Noguchi has called attention to the fact that protein constituents of certain aqueous or alcoholic extracts may have the power to fix complement through certain intermediaries existing in fresh serum which, however, does not obtain for inactivated sera (sera heated to 56° C. for 15 minutes).

Pure lipoidal substances as contained in Noguchi's acetone insoluble antigen, however, do not act in this way.

Consequently by using such an antigen we eliminate the objection to employing fresh human serum in the test for syphilitic antibodies.

As giving more uniform hemolytic results and as being more stable and easier of employment, I have made use of Noguchi's directions for taking up the serum of the rabbits, immunized to human red cells and his method of standardizing this "amboceptor" paper. In practice, I measure off the length of paper corresponding to 8 to 10 Noguchi units and dissolve the dried serum in such paper in 1 c.c. of salt solution. This makes a satisfactory and uniform substitute for the sterile immune serum used by Emery. It has been noted that the dried rabbit serum on the paper may contain a certain amount of complement even when several months old, consequently, to avoid confusion, I invariably inactivate this serum paper solution by heating to 56° C. for 5 minutes.

Method: 1. Take blood from the finger or ear in a large Wright U tube (1/4 inch in diameter). Place in 37° C. incubator for 15 minutes (to increase yield of serum) and then centrifuge.

2. Graduate a capillary pipette for 1 volume and 4 volumes.

3. Into each of a series of small test-tubes put 4 volumes of normal salt solution. (These tubes are most conveniently made by breaking off 2 1/2 to 3 inch lengths of 1/4-inch soft glass tubing and then fusing one end in the flame to make a small test-tube.)

Make a distinguishing mark, *e. g.*, X, on end of tube with blue-wax pencil and use this tube to hold control. Mark the other tubes I, II, III, and so on. When different sera are to be tested they may be distinguished by lines either above or below, or with circles, also marks with red-wax pencil may be used.

4. Make a 1 to 10 dilution of stock antigen solution in salt solution.

To Tube I add 4 volumes of 1 to 10 antigen, thus making 8 volumes of 1 to 20 antigen in Tube I. Mix thoroughly by manipulating bulb of pipette. Then transfer 4 volumes of the 1 to 20 from Tube I to Tube II, and so on through the series. When the dilution in the last tube has been made throw 4 volumes away.

The 4 volumes of dilution of the antigen in the respective tubes will then be: In Tube I, 1 to 20; in Tube II, 1 to 40; in III, 1 to 80; in IV, 1 to 160, and so on.

5. Add 1 volume of serum to be tested to control tube X, and to each of the tubes I, II, III, etc., in succession. (If the serum be added to the antigen tubes before the control tube, antigen might be carried over to the control.)

6. If the serum has been inactivated restore complement by adding 1 volume of a 40% fresh guinea-pig serum. Also use 2 volumes of this inactivated human serum instead of 1.

7. Incubate at 38° C. for 30 minutes. This allows syphilitic antibody, if present, to bind complement.

8. As soon as the above mixtures have been made and put in the incubator prepare the "hæmolytic system" by adding 1 volume of 20% emulsion of washed human red cells to 4 volumes of solution of amboceptor paper (8 to 10 Noguchi units of amboceptor paper dissolved in 1 c.c. of salt solution and then heated to 55° C. for 5 minutes makes a suitable amboceptor solution—thus of a paper of which 4 mm.

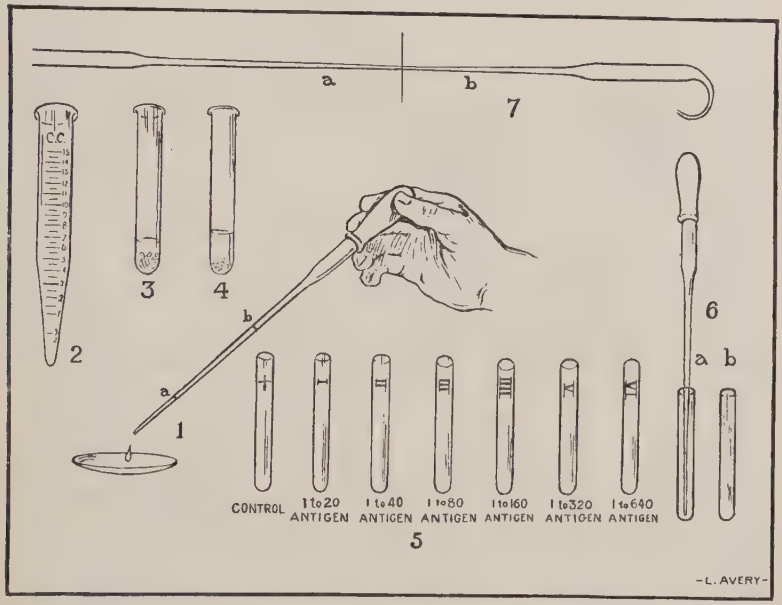

FIG. 48.—1. Capillary pipette being graduated by drawing up 1 and 4 drops from a watch-glass. (a) Blue pencil mark of 1 drop or 1 volume. (b) Mark of volume of 4 drops. 2. Graduated centrifuge tube containing sodium citrate normal salt solution. 3. Tube with 10 amboceptor units in 1 c.c. of salt solution. 4. Mixture of 1 volume 20% emulsion red cells and 4 volumes inactivated amboceptor solution. 5. Small glass tubes for Emery test. 6. Method of transferring from tube to tube. 7. Making a Wright U-tube—the end "a" to be used as a capillary pipette.

was the unit we should cut off about 40 mm., place in test-tube and extract the dried serum with 1 c.c. of salt solution), and place this hæmolytic system in incubator alongside the tubes already there. To obtain the washed red cells allow 4 to 10 drops of blood to drop into a graduated centrifuge tube containing salt solution to which has been added 1% of sodium citrate to prevent coagulation. After shaking, centrifuge. Pour off supernatant fluid, replace with salt solution, again shake and centrifuge—this sediment of red cells is to be diluted with 4 volumes of salt solution (20% emulsion). (Incubation hastens sensitization of the red blood cells. Agglutination of red cells also occurs.)

9. At the expiration of 30 minutes from the commencement of incubation for complement binding, add 1 volume of hæmolytic system to each of the tubes, I, II, III, etc., in the order of antigen dilution.

10. Finally, after washing pipette in salt soultion, add 1 volume of hæmolytic system to control in tube X. (If the hæmolytic system should be added to the control tube before the antigen tubes, complement from the control tube might be carried over to the antigen tubes.)

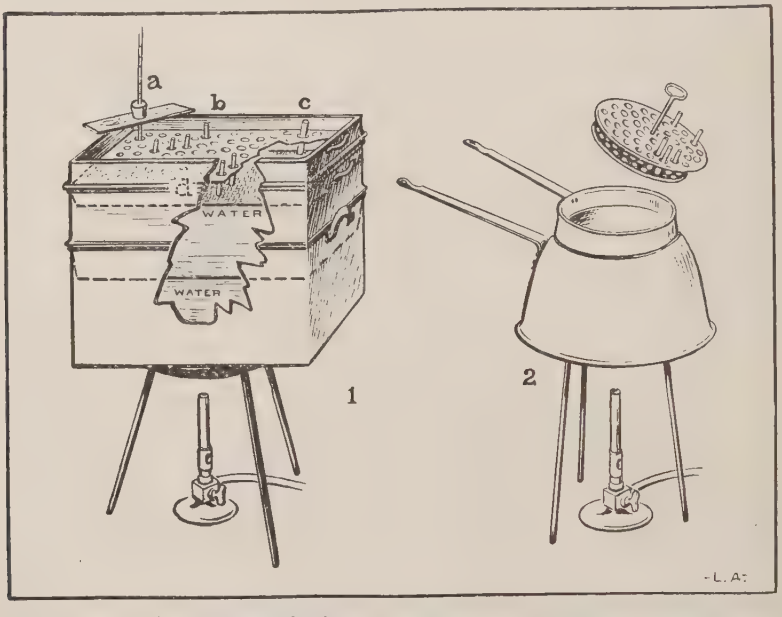

FIG. 49.—1. Copper water bath 12×12×8 inches. (a) Thermometer to show 38° C. (b) Tubes containing antigen dilutions. (c) Tube containing *hæmolytic system* incubating along with the antigen tubes. 2. Ordinary rice cooker with copper holder for test-tubes.

Shake each tube thoroughly. Allow them to incubate for a few minutes. Then examine tubes I, II, III, etc., for hæmolysis. The control should, of course, show hæmolysis. The antigen tubes should show a white, supernatant fluid over the intact red cell sediment in the tubes with the low dilutions and even in the highest dilutions, where the serum is strongly positive. In a weakly positive serum, inhibition of hæmolysis may only show in the first two tubes and hæmolysis show in those tubes having higher dilutions of antigen.

It will be noted that the reagents are made in accordance with Noguchi's directions. Even in those cases where fresh guinea-pig serum is employed to replace complement, absent from the human serum tested, we employ the 40 % solutions used in Noguchi's tech-

nic. It is possibly better to start with a 1–30 dilution in the first antigen tube instead of with a 1–20. It is also an advantage to titrate the human complement.

Preparation of Acetone Insoluble Antigen.—Take about 50 grams of finely divided beef, dog, or rabbit heart or liver and triturate in a mortar to a paste. Pour on this paste 500 c.c. of absolute alcohol and keep the mixture in a corked bottle in the 37° C. incubator for five to seven days. (We use beef heart and 96% alcohol.) Next filter through paper and collect the filtrate in a large shallow dish and hasten evaporation with the aid of a current of air from an electric fan directed upon the uncovered surface.

Within twenty-four hours only a sticky residue should remain. This is taken up in about 50 c.c. of ether and the turbid ethereal solution kept over night in the refrigerator in a corked bottle.

In the morning there will be found about 45 c.c. of clear supernatant fluid which is decanted off and allowed to evaporate to about 15 c.c.

Now to this 15 c.c. add about 150 c.c. of acetone and a precipitate will form which collects at the bottom of the measuring cylinder. Now pour off the supernatant acetone and let the sediment stand until it is of a resinous consistence. Now dissolve 0.3 grams in 1 c.c. of ether and then add 9 c.c. of methyl alcohol. This gives the stock antigen solution.

In using the antigen solution for the Emery or Noguchi test we dilute 1 c.c. with 9 c.c. of salt solution. This opalescent, working, antigen emulsion should be made up fresh on the day of preparing the tests.

About one-half of these antigens are lacking in power to absorb complement in the presence of syphilitic sera. More rarely they may absorb complement with a nonsyphilitic serum (anticomplementary) or they may have a hæmolytic action. Consequently a new stock antigen should be tested as to its reliability—

1. A mixture of 0.4 c.c. working antigen emulsion, 0.6 c.c. salt solution, and 0.1 c.c. of a 10% suspension of washed red cells when incubated at 37° C. for two hours should not show any hæmolysis.

2. A mixture of 0.4 c.c. working antigen emulsion, 0.6 c.c. salt solution, 0.1 c.c. of a 40% solution of fresh guinea-pig serum, and 2 units of amboceptor and incubated at 37° C. for one hour should show hæmolysis when we now add 0.1 c.c. of a 10% washed red-cell emulsion and the whole then again incubated for two hours at 37° C. (The antigen did not absorb complement in the absence of syphilitic antibodies.)

3. A mixture of 0.2 c.c. of a 1 to 10 dilution of working antigen emulsion, 0.8 c.c. of salt solution, 1 drop of syphilitic serum, 0.1 c.c. of a 40% dilution of fresh guinea-pig serum, and 2 units of amboceptor should be incubated at 37° C. for one hour. When we then add 0.1 c.c. of a 10% suspension of washed red cells and again incubate for two hours we should fail to obtain hæmolysis. (The antigen can absorb complement through the intermediation of syphilitic antibodies.)

Preparation of Amboceptor Paper.—In order to secure blood from the vein of a

man or the heart of the immunized rabbit, the most convenient method is with the use of an Erlenmeyer flask with a rubber stopper having two perforations in the stopper. To one of the projecting pieces of glass tubing a stout hypodermic needle is attached through the medium of about 8 inches of rubber tubing, and the second piece of glass tubing is bent at an angle as it leaves the stopper to provide a suction tube. With a man, constrict the upper arm sufficiently to stop venous return with an Esmarck rubber bandage or a towel. Paint tincture of iodine over a prominent vein at the bend of the elbow. Gentle suction will cause the blood to flow into the needle tube and thence into the flask.

The blood as it is taken from the arm should be received in about 50 c.c. of normal salt solution containing 1% of sodium citrate. About 28 to 30 c.c. are usually sufficient. Now throw down this red-cell suspension in three or four centrifuge tubes. The resulting sediment should be washed and rewashed with salt solution. Two to three washings with salt solution suffice.

Now take a large healthy rabbit, shave the lower abdomen and paint the surface with tincture of iodine. The easiest way to inject the rabbit is to hold the animal head down and plunge the needle of a large glass hypodermic syringe containing the washed red-cell sediment into the abdominal cavity in the median line. The intestines gravitate downward and by entering the needle below the limits of the bladder we avoid injuring any vital part.

Make the injections at intervals of five days and give increasing amounts at each successive injection. Thus, first injection, 5 c.c.; second injection, 8 c.c.; third injection, 10 c.c.; fourth injection 12 c.c.; and at the fifth injection give about 15 to 20 c.c. of washed red-cell sediment. It is well to dilute the cell sediment with an equal amount of salt solution. About ten days after the last injection, we take some blood in a Wright's tube from a vein of the ear and dilute the serum to make a 1 to 100 dilution. To 1 c.c. of a 1% emulsion of red cells we add 0.1 c.c. of a 20% dilution of guinea-pig's fresh serum—similar combinations being made in a series of 10 tubes. To each of these tubes we add varying amounts of the 1 to 100 dilution, 0.1 c.c. in the first, 0.2 c.c. in the second, 0.3 c.c. in the third, and so on. If we obtain hæmolysis in the tube containing 0.2 c.c. of 1 to 100 dilution of serum but not in that containing 0.1 c.c. we note that the serum has a titre of about 1 to 500. If the 0.1 c.c. gave hæmolysis, the serum would have a titer of 1 to 1000.

Having ascertained that the hæmolytic serum is sufficiently strong we shave the left side of the thorax of the rabbit and enter the needle of the apparatus similar to that used for taking the blood from a man's vein in one of the intercostal spaces of the left side.

Having introduced the needle, feel for the heart beat and then plunge the needle into the heart. We can withdraw about 30 c.c. of blood without injury to the rabbit. This blood should be received in a clean empty flask and set, over night, in the refrigerator. The following morning pour off the clear serum into a clean Petri dish and saturate, one by one, squares of filter paper with the serum. Allow the filter paper to dry on a piece of unbleached muslin. Noguchi recommends Schleich and Schull's paper No. 597. When thoroughly dry cut strips 10 mm. wide. This makes the amboceptor paper. To standardize, take a series of tubes containing 1 c.c. of a 1% emulsion of red cells and add 0.1 c.c. of 20% dilution of guinea-pig serum for complement. Next cut across the amboceptor paper strip pieces of varying width,

as 1 mm., 2 mm., 3 mm., 5 mm., and so on. The narrowest strip which gives hæmolysis in one hour equals one unit. Thus if a piece 5 mm. wide was required to produce hæmolysis, 5 mm. of the paper would have a value of one unit.

Noguchi's Method

For the suspension of red cells use a 1/2% suspension of washed human red cells.

For complement use fresh guinea-pig serum in a dilution of 1 part to 1 1/2 parts of salt solution (40%).

Experiment.—Take 4 small test-tubes (12 by 125 mm.) label *1a*, *1b* and *2a*, *2b*, respectively. Into *1a* and *1b* each put 1 drop of the serum of the patient to be tested and into *2a* and *2b* each put 1 drop of the serum of a person known to give a positive test for syphilis. Next add to each of all four tubes 1 c.c. of the 1/2% suspension of washed red cells. Then add to each tube 0.1 c.c. of the 40% fresh guinea-pig serum. Now add to tube *1a* and tube *2a* each 0.1 c.c. of the 1 to 10 antigen dilution (opalescent working antigen emulsion). Tubes *1b* and *2b* are controls not containing antigen. Mix contents of tubes thoroughly and incubate at 37° C. for one hour or for 1/2 hour in a water bath. Now add to each of the four tubes 2 units of the immune hæmolytic serum, as measured off on the amboceptor paper strip—thus with a paper of which 2 mm. equals 1 unit, drop into each tube 4 mm. of the strip.

The tubes without antigen (*1b* and *2b*) should show good hæmolysis. Tube *2a*, that of the known syphilitic, with antigen, should not show hæmolysis and that of the person examined (*1a*) should show hæmolysis in case the test is negative for syphilis. Moderately positive cases may show a slight trace of hæmolysis. In case the tubes without antigen are negative (no hæmolysis), repeat the test with smaller amounts of human serum. It may be advisable to employ the serum of a person known to be free from syphilis. In this case we should use two additional tubes, *3a* and *3b*, conducting the test as for the syphilitic control serum.

Many workers prefer to use inactivated serum for the test. In this case we should add four times as much of the inactivated serum as for the unheated serum.

Inactivation not only destroys complement but likewise diminishes the strength of the antibody content of the serum. Factors such as character of food and general condition influence the complement strength of guinea-pig serum so that it is advisable to titrate the guinea-pig serum. To do this take 1 c.c. of a 1% emulsion of human red cells and drop in one unit of amboceptor paper. The amount of comple-

ment which will entirely hæmolize the red cells in one-half an hour in water bath equals one unit of complement. For the test use two units of complement.

The Wassermann Test.

In the Wassermann reaction the rabbits are injected with sheep red cells which have been washed twice with salt solution by aid of the centrifuge. About five injections with, on the average, the quantity of red cells contained in 5 c.c. of sheep blood given at intervals of five days gives a strong hæmolytic serum if taken about one week after the last injection. The method is to take in a test-tube 0.2 c.c. inactivated human serum (heated for one hour at 56° C.), 0.1 c.c. fresh serum from guinea-pig for complement, 1 unit antigen and 3 c.c. normal salt solution; then to incubate for one hour at 37° C. (An antigen unit is the amount that will inhibit hæmolysis of 1 c.c. of 5% emulsion of sheep cells when mixed with 0.2 c.c. luetic serum and 0.1 c.c. guinea-pig complement.) Then add 2 units of amboceptor and 1 c.c. 5% emulsion of sheep red cells, shake and incubate for one hour. (The amount of hæmolytic serum that will hæmolize 1 c.c. of a 5% emulsion of sheep red cells to which 0.1 c.c. guinea-pig serum has been added, in one hour, is an amboceptor unit.)

The same technic is employed with the control test-tube except that the antigen unit is not put in.

The Noguchi method gives a positive reaction with nonsyphilitic sera in about 7% of cases. The Wassermann gives a negative result in about 9% of syphilitic sera. These figures show the advantage of checking one against the other.

There seem to be certain sera when with a clinical history of syphilis we obtain a positive Wassermann with unheated serum and a negative one with inactivated serum. In order to obtain information with the same serum heated and unheated I would recommend, when it is inconvenient to carry out the original Wassermann technic, to employ the Noguchi technic with inactivated serum and the Emery technic with fresh unheated serum. In any case when serum cannot be tested within twenty-four hours it should be inactivated, as unheated serum tends to become anticomplementary.

Cherry thinks anticomplementary bodies are found during chloroform anæsthesia. If the antigen should also have anticomplementary action the total might give a negative result.

By heating the serum for half an hour at 56° C. (inactivation) the positive results obtained in certain cases of cancer, nephritis, scarlet fever, leprosy and tuberculosis may be avoided; the syphilitic antibody alone being thermostable. The

thermostability of serum of inherited syphilis is the highest—that of primary syphilis the least of luetic sera.

McDonagh states that in the primary stage the Wassermann is positive in 40% of cases. In secondary cases 97% give positive results when treatment has not been instituted. In tertiary syphilis about 70% are positive.

In 268 cases at the medical clinic of Johns Hopkins Hospital, Clough failed to obtain a positive reaction in ninety-nine cases which were negative clinically.

In forty-five cases of syphilis he obtained 73% of positive results. Excluding cases which had received thorough treatment 82% were positive. Tabes gave 40% and general paresis 100 %. In five cases of primary syphilis four gave positive reactions.

Based upon the observation of Bauer, that human serum contains hæmolytic amboceptors for sheep corpuscles, and of Hecht, that the complement normally present in human serum would suffice without the addition of guinea-pig serum complement, the following method of *Fleming* is easy of application.

For the test we use:

1. Alcoholic extract of rabbit's heart, made by washing the recently removed heart with salt solution to remove all blood. Cut into small pieces and grind in a mortar with sand and for every gram of heart add 5 c.c. of 95% alcohol. Keep the mixture at a temperature of 60° C. for two hours and filter. This is the stock solution. For use dilute it ten times with normal salt solution.

2. A 5% emulsion of washed sheep red cells, prepared as for the Wassermann test.

3. Suspected and control sera.

With a capillary bulb pipette take up one part of serum and four parts of the heart antigen, mix on a glass slide, again draw up into the capillary pipette and, leaving a separating air space, next draw up one part of 5% emulsion of sheep red cells. Then seal off tip of pipette and incubate at 37° C. for one hour. Now file off tip and mix the red cells with the serum and antigen and again draw up into the capillary pipette and incubate a second time for two hours. Hæmolysis or the reverse is shown in the fluid overlying the cell sediment. Various controls should be made using normal and known syphilitic sera; also with normal salt instead of serum.

The objections to methods using human serum for complement are 1. the great variation in the complement content of different human sera; 2. human complement requires about ten times as much amboceptor as guinea-pig complement and is less sensitive to fixation, and 3, the statement is made by some workers that while homologous complement and amboceptor may be efficient yet the complement of a

serum will not act upon its homologous antigen. This is not true because the complement of human serum invariably hæmolyzes the homologous antigen (human red cells).

The various precipitate tests that have been proposed are unreliable. The precipitate reactions with bile salts give better results than with lecithin, this latter showing positive results in almost one-half of non-syphilitic cases.

Determination of Opsonic Power and the Preparation of Vaccines.

In that which has been considered in the previous pages only the theories of Ehrlich have been brought out. In order to understand the problems involved in the study of opsonins the phagocytic theory of immunity brought forward by Metchnikoff must be studied. Ehrlich's views would seem to hold with diseases where there is an increase in bacteriolytic or antitoxic power of the serum while in such diseases, as are caused by pathogenic cocci, the phagocytic element is operative as there is an absence of bacteriolytic power in the serum of the person with the infection.

There are two kinds of phagocytes, the microphages (represented by the polymorphonuclears) which on phagolysis or disintegration give off microcytase, a bactericidal substance. Cytase is the same as complement or alexine.

The microphages are chiefly bactericidal while the macrophages, represented by the large mononuclears of the blood and fixed connective-tissue cells, exert their action on protozoa or animal cells.

Phagocytes may either act by ingesting bacteria and destroying them intracellulary or they may as a result of phagolysis bring about bacteriolysis extracellularly. According to Metchnikoff the intracellular bacteriolysis explains why an individual may possess immunity and yet his serum fail to show any bacteriolytic power.

The following modification of Leishman's method takes very little time and skill and is applicable in the determination of the organism concerned in an infection, as in Wright's method. The control of vaccine treatment by taking opsonic indices from time to time does not seem to have met with much favor in this country—the sources of error being as great, if not greater, than ordinary variations in the opsonic index during the negative and positive phases.

Method.—We start with a 1% solution of sodium citrate in salt solution. With this emulsify a twelve to twenty-four-hour agar slant growth of the organism to be tested using 6 to 8 c.c. of the citrated salt solution. The bacterial emulsion is now

poured into a bottle or sealed off in a test-tube and shaken thoroughly in a shaker or by hand. The emulsion is then centrifuged to throw down the bacterial clumps and the supernatant slightly turbid bacterial suspension poured off. If working with a dangerous pathogen it is advisable to kill the organisms as in making vaccines.

Now with a capillary bulb pipette so graduated that the one volume mark contains about two drops we draw up one volume of citrated salt solution. Then having made a break with an air column, we take up one volume of the patient's blood. Again make an air break and draw up one volume of the citrated salt solution bacterial emulsion. The three volumes are then immediately forced out into a small test-tube, made from three inches of 3/16-inch glass tubing, as shown in the Emery technic for the Wassermann. The citrate prevents coagulation of the blood and the contents of the tube are well mixed by drawing up and ejecting with the capillary bulb pipette. Incubate this small test-tube at body temperature for 15 minutes, shaking the contents once or twice during the incubation period. Exactly at the expiration of the period of incubation (usually 15 minutes although at times 10 minutes or 30 minutes may be desirable) place the tube in a centrifuge and throw down the cell sediment. Next pipette off the supernatant fluid and then plunge the pipette to the bottom of the tube and draw off the greater part of the sediment at the bottom. This consists largely of the red cells the leukocyte layer on the surface being undisturbed.

Now mix the remaining cell sediment and smear out on a slide or preferably between two cover-glasses as in Ehrlich's method. The smear is fixed by burning off a film of alcohol and stained with dilute carbol fuchsin or methylene blue. The granule staining with Wright's stain makes it slightly confusing.

A second similar preparation but using blood from a normal person as a control is then made. Counting the phagocytized bacteria in a given number of polymorphonuclears, we obtain an average number of bacteria phagocytized per cell. Repeating the count with the control or normal blood, we likewise have the average number of bacteria taken up per cell. Dividing the patient's average by the normal average, we have the opsonic index. If the average for fifty of the patient's cells was eight and that of the control only four, the patient's index would be two, or twice the normal. The practical value of this test is that where two or more organisms are in a body fluid we may ascertain the causative organism by noting marked variation from the normal in the patient's opsonic index for that particular organism and not for the other organism. This variation may be of the nature of a high or low opsonic index.

Method of Wright for Obtaining Opsonic Index.

While other observers had previously noted the presence of substances in immune sera which so acted on the bacteria that phagocytosis was made possible, yet it was to Wright and Douglas, in 1903, that the existence of this factor in phagocytosis was brought forward and the estimation of such substances made practicable.

To this substance the name opsonin was given—the Greek word

from which it is derived indicating preparation of the food—that is, the opsonin so alters or sensitizes the bacteria that they can be engulfed or phagocytized by the polymorphonuclear leukocytes (the microphages of Metchnikoff). About the same time Neufeld and Rimpau noted the presence of a substance in immune sera which so acted on bacteria as to prepare them for phagocytosis. Their designation "bacteriotropic substance" is practically synonymous with opsonin.

In 1902 Leishman introduced the method of determining the "phagocytic index." By taking one part of blood and one part of an emulsion of the bacteria in question and keeping the mixture in a moist chamber at body temperature for a standard time, as 15 to 30 minutes, and then spreading the blood-bacteria mixture and staining the film with Leishman or Wright's stain he counted the number of bacteria in a certain number of polymorphonuclears, and by dividing obtained the average number per leukocyte of bacteria phagocytized.

The Wright technic for determining the phagocytic average, and from this the opsonic index, is as follows:

Blood is taken from the patient and at the same time from a normal individual, or preferably the blood of several normal individuals is pooled. This blood is best collected in a Wright's tube, although it may be received in a small test-tube. After coagulation and separation of the serum, the serum is ready for use.

The next step is to prepare the leukocyte emulsion. For this we fill a centrifuge tube with normal salt solution, to which has been added 1% sodium citrate —the latter to prevent coagulation. Then having pricked a finger congested by a constricting rubber band, from 15 to 20 drops of blood are added to the citrated salt solution, and the mixture thoroughly shaken. After centrifugalization for about 5 minutes the red corpuscles will be thrown to the bottom of the tube with the leukocytes forming a superimposed layer. In order to free the leukocytes entirely from serum admixture, the supernatant citrated salt solution is pipetted off, and a fresh tubeful of salt solution is added to the blood-cell sediment. Again shaking, we then centrifuge, obtaining for a second time a sediment of blood cells with the leukocytes in the superimposed layer. In some laboratories the washing in salt solution is again repeated, but for all practical purposes two washings as described above suffice.

The superimposed layer of white cells may now be pipetted off from the heavier red cells (of course, containing a large admixture of red cells) to be used as a leukocyte cream—or by slanting the centrifuge tube we can pipette off the proportion of the leukocyte mixture needed from the bottom, sides or top of the slanted layer of blood cells.

Having prepared our leukocyte emulsion, and the serum from the normal individual as well as that from the patient, it only remains to prepare our bacterial emulsion. For bacteria in general, with the exception of tubercle bacilli, we simply take up a small loopful of a young agar culture (eighteen hours or less), and emulsify it uniformly with salt solution, added by degrees until the suspension amounts to 1/2 to 1 c.c., and giving a faint turbidity. To thoroughly distribute and especially

to break up clumps repeated suction and ejection with a capillary pipette provided with a rubber nipple is satisfactory.

The presence of clumps in a bacterial emulsion invalidates the estimation of phagocytosis, for the reason that a leukocyte will take up a clump of twenty or more bacilli as readily as one separate organism.

Having at hand (1) the suspension of leukocytes, (2) the bacterial emulsion, and (3) the sera of the patient and the normal individual, we are ready to proceed with the test.

Using a capillary bulb pipette with a pencil mark to indicate 1 volume we draw up to the mark (1) the leukocyte cream. Then wiping off the tip of the pipette we draw up this volume of leukocyte emulsion about one-half an inch to make an air break between this and (2) 1 volume of the bacillary emulsion. Again making an air space we draw up (3) the serum of the normal individual. This gives 3 columns in the capillary tube with intervening breaks of air. We next eject the three constituents into a watch-glass and thoroughly mix them by alternate suction and ejection with the tube and nipple. When mixed we draw the mixture up into the same capillary tube, seal off the capillary end in the flame and put in an incubator for exactly 15 minutes.

We next repeat the process identically except that the patient's serum is used instead of that of the normal individual.

These tubes having been kept at the same temperature for the same length of time are then taken out, the contents blown into a watch glass, mixed thoroughly a second time, and then a smear is made—a drop of the mixture being deposited on a very clean slide and the smear made by a second narrower slide (by cutting off the corner of the slide) which is drawn along in a zigzag way. The smears are then stained (Leishman's or Wright's blood stain or Ziehl-Neelson's for tubercle bacilli) and the number of the bacteria in from fifty to one hundred leukocytes counted. This number divided by the number of cells gives the phagocytic average.

The phagocytic average of the patient's tube divided by that of the normal individual's tube gives the opsonic index. Thus, in counting 100 cells we find 500 phagocytized cocci in the patient's tube, giving an average of 5, and in the normal individuals blood we get 1000, an average of 10. Then the opsonic index would be $5 \div 10$, or 0.5.

Preparation of Vaccines.

It has been found satisfactory to make use of stock vaccines in gonorrhœal and tuberculous affections. In treatment of tuberculosis Wright prefers Koch's T. R. or Neu Tuberculin in doses of from 1/5000 to 1/800 of a milligram. Some prefer Koch's more recent bazillen emulsion. In case of other infections, however, and preferably with gonorrhœal infections, the causative organism should be isolated from pus, sputum, urine, blood, or other material (autogenous vaccine).

In the making of vaccines all media and apparatus should be sterilized with scrupulous care to avoid the danger of tetanus infection. Having isolated the organ-

ism, it is inoculated upon one or more agar slants, and after a growth of from five to seven hours with streptococci and pneumococci, or with eighteen hours for staphylococci and colon, the growth on these inoculated slants is taken up with salt solution, thoroughly shaken up in the diluting solution and standardized.

The most practical way is to gently rub off the growth on the agar in about 1 or 2 c.c. of salt solution with a platinum loop. Then pour the bacterial emulsion into a sterile test-tube and repeat the process with three to five agar slants, until we have from six to 10 c.c. of the emulsion in the sterile test-tube. By heating to melting-point in the flame a piece of glass tubing and attaching it to the rim of the test-tube (also melted), we have a handle with which to draw out the test-tube when heated about 1 inch from the mouth in a blowpipe flame. Drawing this out, we let it cool, and then filing the constricted portion we break it off and seal it in the flame. By shaking up and down vigorously for five to fifteen minutes, or preferably in a mechanical shaker the bacteria are distributed evenly in the salt solution. A piece of platinum wire, twisted into corkscrew shape, and fused in the drawn out end of the containing test-tube helps in breaking up the bacterial emulsion and is a great aid in the preparation of streptococcic or diphtheroid vaccines.

The sealed test-tube is then placed in a water-bath at 60° C. and heated at this temperature for one hour. Again shake. The constricted sealed end is again filed off and a few drops shaken out in a watch glass for standardization, and at the same time a few drops are deposited on an agar slant as a test for sterility. (Incubation for twenty-four hours should not show growth.)

Wright found that by taking a definite quantity of blood and a similar quantity of bacterial emulsion, mixing the blood and bacterial emulsion, then making a smear and staining, it was possible to determine the ratio of bacteria to red cells, and from this the number of bacteria per cubic centimeter could be determined. For example, if we find three bacteria to each red cell we should have 15,000,000 bacteria to 1 cubic millimeter. (There being 5,000,000 red cells to the cubic millimeter.) As 1 cubic centimeter is 1000 times greater than 1 cubic millimeter, there would be 15,000,000,000 bacteria in each cubic centimeter of such an emulsion, or vaccine, as it is termed.

The standardization made with a hæmacytometer is best done by drawing up the vaccine to 0.5 with either the red or white pipette, according to concentration, and then sucking up 1 to 10 dilute carbol fuchsin to 11 or 101. Allow the bacteria to settle on the shelf for ten minutes before counting. Count as in making a red count.

A more satisfactory diluting fluid is that recommended by Callison. It is: Hydrochloric acid 2 c.c., Bichloride of mercury (1–500 aq. sol.) 100 c.c., and sufficient 1% aqueous solution of acid fuchsin to color the diluting mixture a deep cherry red. The diluting fluid should then be filtered. The bichloride forms an albuminate on the surface of the bacteria which promotes rapid sedimentation and the fuchsin stains the bacteria.

Having determined the strength of the stock vaccine, we should prepare a dilute vaccine for injection. This is most conveniently carried out by filling vials with 50 c.c. of salt solution, plugging with cotton, then sterilizing in the autoclave. A sterile rubber cap is now drawn over the mouth of the vial. Sterility is insured by plunging the rubber cap and neck in boiling water. If the stock vaccine showed 5,000,000,000 bacteria per c.c. and we desired to have a vaccine containing 200,000,000 bacteria per c.c., it would be necessary to draw out 2 c.c. of the salt solution by means of a sterile syringe needle inserted through the rubber cap and replace it with 2 c.c. of the bacterial emulsion. Example: In introducing 2 c.c. of a vaccine containing 5,000,000,000 bacteria per c.c., we throw in 10,000,000,000 bacteria in a volume equal to 50 c.c. Then each c.c. of the 50 c.c. in the bottle would contain 10,000,000,000 divided by 50 or 200,000,000 in each c.c. If we only want a vaccine containing 100,000,000 per c.c. we should only add 1 c.c. We now add 1/4% of trikersol to the vaccine in order to insure sterility. (Introduced with syringe, inserting needle through rubber cap.) The syringe is best sterilized by drawing up vaseline or olive oil heated to 150° C., and the neck and rubber cap of the bottle in boiling water. We now draw up the desired dose of bacteria. If glass syringes are used, simply boiling in water suffices. The ordinary doses are: For gonococci, streptococci, pneumococci, and colon vaccines, 5,000,000 to 50,000,000. For staphylococci 200,000,000 to 1,000,000,000.

Wilson gives the following minimum and maximum doses expressed in millions:
Streptococcus, 6 and 68.
Gonococcus, 45 and 900.
Meningococcus, 300 and 900.
M. melitensis, 700 and 1400.
B. coli, 16 and 240.
B. typhoid (treatment) 100 and 250.
B. typhoid (prophylaxis) 500 and 1000.
B. pyocyaneus, 34 and 1000.
B. pneumoniæ, 44.
Staphylococci, 150 and 900.
B. tuberculosis, 1/20000 to 1/200 milligram.

Anaphylaxis.

This is a term which indicates the opposite of prophylaxis. It was noted that after a period of incubation of at least ten days a second

injection of horse serum produced symptoms of respiratory embarrassment, convulsions and, at times, death. The primary injection had during the period of incubation sensitized the cells to this particular proteid.

This phenomenon of sensitization in the case of rabbits bears the name of Arthus, and as applied to guinea-pigs sensitized with diphtheria antitoxin sera the name Theobald Smith, and it is stated by Muir and Ritchie that active research as to anaphylaxis may be said to date from the discovery of the phenomenon of Theobald Smith.

Rosenau and Anderson working with guinea-pigs showed that small doses were efficient for sensitization, that the condition was transmissible from mother to offspring and that a second animal could be sensitized by being injected with the serum of a sensitized animal.

This group of symptoms, the so-called anaphylactic shock, which is apt to set in within a few minutes after the second injection, is often preceded by restlessness and great excitement and together with the dyspnœic manifestations there is cardiac weakness and great fall of blood-pressure. The more serious symptoms and at times death are more apt to appear after intracerebral injections than after intraperitoneal. Subcutaneous injections are least apt to produce anaphylactic symptoms. Our attention to this phenomenon commenced with the study of "serum sickness" or "serum disease." In this an erythematous rash or urticaria associated with more or less œdema comes on after eight to twelve days from the time of the first and only injection of horse serum. It is supposed to be due to the fact that some of the serum originally injected remains unchanged in the tissues so that when the sensitization takes place there is present and at hand the same foreign proteid to bring about anaphylactic symptoms.

Immunization against anaphylaxis is possible by repeating injection of the sensitizing serum or proteid during the period of incubation.

It is important to note that this hypersusceptibility appears to be very rarely of importance in the matter of the administration of a second injection of diphtheria antitoxin after the period of anaphylactic incubation.

As a rule the death or untoward effects of the injection of serum are in cases of status lymphaticus. Cases in man do occur, however, but with extreme infrequency, in which within a few minutes after the only injection of serum the patient becomes restless, shows symptoms of cardiac and repiratory embarrassment and may be dead in a very short time.

According to Rosenau and Anderson individuals who have asthmatic tendencies as well as those who have had serum injections ten to twelve days or longer prior to

the second injection should be considered as possible subjects for anaphylactic shock.

Vaughan recommends that when this is to be feared one should only give about 0.1 c.c. of the serum and after an interval of two hours, provided no untoward symptoms set in, to give the full amount of the injection. Besredka advises heating the serum to 56° as a guard against anaphylactic shock.

The condition of hypersusceptibility or anaphylaxis is at times termed allergy. Thus in a person who has been successfully vaccinated a reaction shows at the site of inoculation within twenty-four hours which does not appear in the nonimmune person for a period two or three times as long. The diagnostic tests with tuberculin and luetin are hence often referred to as allergic reactions.

It may here be stated that some investigators are of the opinion that our views not only as to immunity but as to the essential nature of infectious diseases may be later on found to rest in production of anaphylaxis.

The name "Anaphylactine" has been applied to the sensitizing substance produced during the period of incubation.

It has been proposed to employ this phenomenon as a diagnostic measure. By taking the serum of a tuberculous patient, which would contain the sensitizing substance, and injecting it into the peritoneal cavity of a rabbit, the animal would be sensitized and an injection of tuberculin a few hours later would bring about the phenomena of anaphylaxis in the rabbit.

This passive anaphylaxis, as it is termed, usually requires approximately twenty-four hours for sensitization. This passive anaphylactic sensitization seems to disappear in two weeks. It has been advised to passively sensitize guinea-pigs with the serum of the person about to be injected and then twenty-four hours after inject the guinea-pigs with the curative serum. If untoward results occur in the guinea-pigs the patient should not receive the injection.

Recently Hagemann has found the following technic valuable in the diagnosis of surgical tuberculosis. Guinea-pigs are inoculated intraperitoneally with tuberculosis cultures and by the end of the second week such pigs are sensitized. The suspected material, as serous effusion, is injected intracutaneously and within twenty-four to forty-eight hours a distinct swelling of the skin with a bluish-red center, which is surrounded by a porcelain white ring and outside of this a zone of inflammation, shows a positive test.

PART II.

STUDY OF THE BLOOD.

CHAPTER XIII.

MICROMETRY AND BLOOD PREPARATIONS.

Micrometry.

In the examination of blood and fæces preparations, especially when the identification of animal parasites is in question, there is nothing that assists more than a knowledge of the measurements of the object studied. The making of such measurements microscopically is termed micrometry.

Micrometry is also indispensable in bacteriology and cytodiagnosis as well as in animal parasitology.

The most practical way of making these measurements is with an ocular micrometer. These can be bought separately, or a glass disc (disc micrometer) with lines ruled on it can be dropped into the ocular to rest on the diaphragm inside the ocular. The ruled surface of this glass diaphragm should be placed downward. As was stated in connection with the microscope, the image of the object is formed at the level of the diaphragm rim inside the ocular, consequently the lines of the image cut those of the lines ruled on the glass in the ocular. Once having standardized the value of the spaces of the ocular micrometer for each different objective, all that is necessary subsequently in measuring is to count the number of lines or spaces which the image of the object fills and then, knowing the value of each space for that objective, to multiply the number of spaces by the value of a single space.

The unit in micrometry is the micron. This is usually written μ and is the 1/1000 part of a millimeter. There are 1000 microns in a millimeter.

To standardize: For this purpose it is necessary to have a scale of known measurements. The stage micrometers are usually ruled in spaces of 0.1 and 0.01 mm. The lines which are 1/10 of a millimeter apart are consequently separated by a distance of 100 microns; those 1/100 of a millimeter apart are separated by a distance of 10 microns.

The ocular micrometer is usually ruled with 50 or 100 lines or spaces, separated by longer lines into groups of 5 and 10.

Having brought the lines on the stage micrometer to a focus, we determine the number of spaces on the stage micrometer which the 50 divisions of the ocular micrometer cover. To distinguish the ruling of the ocular from that of the stage micrometer, revolve the ocular with the fingers.

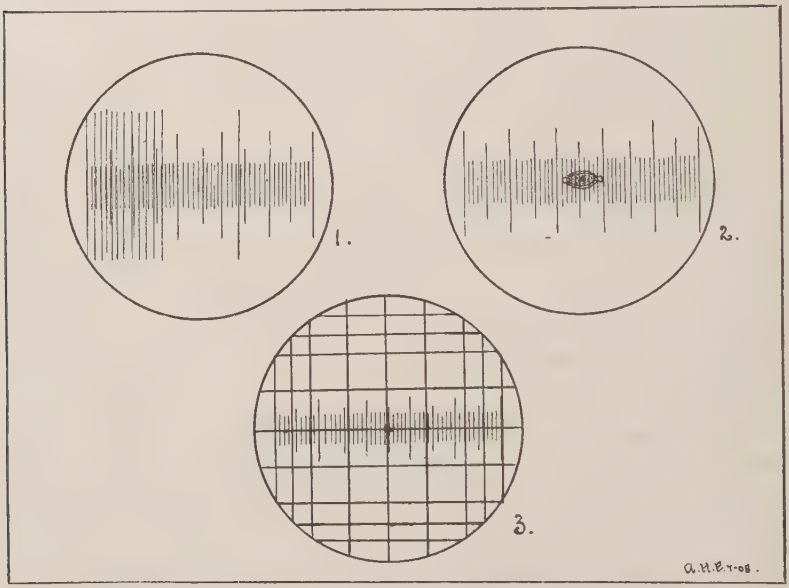

FIG. 50.—Micrometry diagrams. 1. Ocular micrometer with stage micrometer. 50 spaces of ocular micrometer cover two 100 micron spaces and ten 10 micron spaces; equal 300 microns. Each division on ocular micrometer equals 6 microns. 2. Ocular micrometer subtending image of whip worm egg. 9 spaces of ocular micrometer cover Whipworm egg. Each space equals 6 microns. Whipworm egg equals 54 microns. 3. Ocular micrometer with ruling of hæmacytometer. 50 spaces of ocular micrometer cover space equal to width of 6 small squares $50 \times 6 = 300$ microns. Each division of ocular micrometer equals 6 microns.

The tube length which is used at the time of standardizing must always be adhered to in subsequent measurements.

Example: With a 2/3-inch objective, the 50 rulings of the ocular micrometer fill in fifteen of the 1/10 millimeter rulings (100μ) and three of the 1/100 millimeter spaces (10μ). Consequently the 50 spaces of the ocular cover 1530 microns ($15 \times 100 = 1500$; $3 \times 10 = 30$). Then if 50 spaces equal 1530 microns, one space would equal

30.6 microns. With the 1/6-inch objective the 50 ocular spaces would cover about three of the 1/10 millimeter (100μ) spaces of the stage micrometer. Then the 50 spaces would equal 300 microns and one space would equal 6 microns.

The ruling of the slide of a Thoma-Zeiss hæmocytometer will answer as well as a stage micrometer. The small squares are 1/20 of a millimeter square, consequently the distance between the lines bordering the small square is 1/20 millimeter or 50 microns.

Now, if with the 1/6-inch objective, the 50 lines on the ocular fill in the spaces of six small squares, the length of such a space would be $50 \times 6 = 300$ microns. This divided by 50 spaces would equal 6μ.

Should there be 100 spaces on the ocular micrometer instead of 50, it would only be necessary to divide the length in microns of the ruled surface of the stage micrometer covered by the 100 lines of the ocular micrometer by 100. The quotient would give the value in microns of each space of such an ocular micrometer.

The most accurate instrument for measuring is the filar micrometer. These are expensive. Measurements can also be made with the camera lucida, but it takes considerable time to make the adjustments necessary, so that it is not convenient. With an ocular micrometer one can make measurements of blood-cells, amœbæ, etc., in a few seconds—it only being necessary to slip in the ocular micrometer.

Rule for determining the magnifying power of microscopic lenses: Measure the diameter of the lens of the objective in inches—the approximate equivalent focal distance is about twice the diameter. Dividing 10 by the equivalent focal distance gives the magnifying power of the lens. This should be multiplied by the number of times the ocular magnifies. Example: The diameter of the lens of the objective was found to measure 1/2 inch, the focal distance would then be about 1 inch. Dividing 10 by 1 we have 10 as the magnifying power of the lens of the objective. If we were using a No. 4 ocular, the magnifying power would be approximately forty.

Blood Preparations.

To obtain blood, except for blood cultures, use either a platino-iridium hypodermic needle which can be sterilized in the flame, a small lancet, or a surgical needle with cutting edge.

When using such surgical needles it is a good plan to sharpen the cutting edge on a fine-grained whetstone. Afterward the needle should be sterilized by boiling. Sterilization of a needle in the flame blunts the cutting edge. A steel pen with one nib broken off or the glass needle of Wright may also be used. To make a glass needle, pull straight apart a piece of capillary tubing in a very small flame. Tap the fine point to break off the very delicate extremity. Scarcely any pain attends the use of such a needle. In puncturing either the tip of the finger or lobe of the ear a quick piano-touch-like stroke should be used. The ear is preferable, as it is less sensitive and there is less danger of infection. Before puncturing, the skin should be cleaned with 70% alcohol and allowed to dry. It is advisable to sterilize the needle before using it.

The first drop of blood which exudes should be taken up on the paper of the Tallquist hæmoglobinometer, using subsequent ones for the blood pipettes and smears. If it is necessary to make a complete blood examination, it is rather difficult to draw up the blood in the pipettes, dilute it, and then get material for fresh blood preparations and films without undue squeezing, which is to be avoided. Of course, fresh punctures can be made. Ordinarily, complete blood examinations are not called for. It is only a white count or a differential count or an examination for malaria that is required.

As a practical point it is very rare that a red count is indicated. There is one point not sufficiently recognized by physicians and that is that a call for a routine blood examination is not apt to be as carefully conducted as one calling for a specific feature. Without disparaging the necessity of routine examinations of urine as well as blood it is a fact that the internist who knows what he wants gets better results from the laboratory man.

Hæmoglobin Estimation.

The most accurate instrument for this purpose is the Miescher modification of the v. Fleischl hæmoglobinometer.

The magenta-stained glass wedge for comparison with the diluted blood is similar in each instrument, but by the use of a diluting pipette accurate dilutions are possible in the Miescher. There are two cells provided—one 12 millimeters high, the other 15 millimeters; the idea of this being to enable one to make separate comparisons and to select the central part of the glass-wedge scale, where comparison is more accurate than at the ends. As these cells contain columns of diluted blood proportionately as 5 to 4, we should have similar readings when we multiply the reading on the scale with the 15 mm. cell by 4/5.

The mixing pipette is graduated with the marks 1/2, 2/3 and 1/1—the first giving a dilution of 1 to 400 (when the diluent, a 0.1% soda solution, is drawn up to the mark above the bulb) the second of 1 to 300 and the last of 1 to 200.

Artificial light preferably from a candle is necessary. There is a table accompanying each instrument which shows the value for that particular instrument in milligrams per liter of hæmoglobin for any reading obtained on the scale.

The apparatus is expensive, requires considerable time and care in the making of estimations, and is exclusively an instrument for a well-equipped laboratory.

Sahli's Hæmometer.—A simple and apparently very scientific instrument which has been recently introduced is the Sahli modification of the Gower hæmoglobinometer. Instead of the tinted glass, or gelatin colored with picrocarmine to resemble a definite blood dilution, Sahli uses as a standard the same coloring matter as is present in the tube containing the blood. By acting on blood with ten times its volume of

N/10 HCl, hæmatin hydrochlorate is produced, which gives a brownish-yellow color. In the standard tube, which is sealed, a dilution representing 1% of normal blood is used. To apply this test, pour in N/10 HCl to the mark 10 on the scale of the graduated tube. Add to this 20 cubic millimeters of the blood to be examined, drawn up by the capillary pipette provided. So soon as the mixture assumes a clear bright dark brown color, add water drop by drop until the color of the tubes matches. The reading of the height of the aqueous dilution on the scale gives the Hb. reading. The tubes are encased in a vulcanite frame with rectangular apertures. This gives the same optical impression as would planoparallel glass sides.

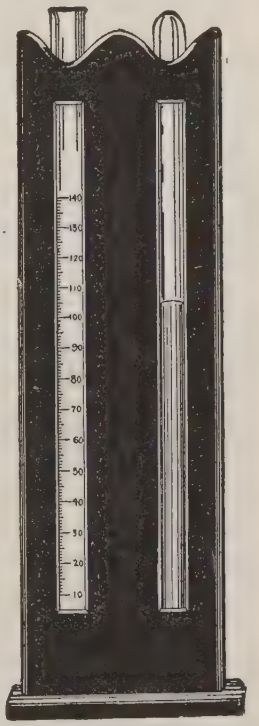

FIG. 51.—Sahli's hæmoglobinometer. (*Greene.*)

The most accurate readings are obtained with artificial light in a dark room but almost as satisfactory comparisons can be obtained with natural light from a window. It is advisable to turn the ruled side around so that one may match colors without being influenced in his determination by the scale.

The apparatus must be kept in a dark place as strong light will change the color of the standard tube. It is recommended that the N/10 HCl be preserved with chloroform.

Tallquist's Hæmoglobin Scale.—This is a small book of specially prepared filter-paper with a color-scale plate of ten shades of blood colors. These are so tinted as to match blood taken up on a piece of the filter-paper and are graded from 10 to 100. So soon as the blood on the filter-paper has lost its humid gloss, the comparison should be made. This is best done by shifting the blood-stained piece of filter-paper suddenly from one to the other of the holes cut in each shade—the piece of filter-paper being underneath the color plate. At least a square centimeter of the filter paper should be stained by the blood. Daylight coming from a window to the rear or at the side should be used in making the comparison. The error with this method is probably not over 10% after a little experience. If the colored plate is not kept in the dark, the tints tend to fade.

To Count Blood-corpuscles.

The instrument almost universally used is the Thoma-Zeiss hæmacytometer. The apparatus consists of two pipettes, one for leukocytes, graduated to give a dilution of 1 to 10 or greater; the other for red cells to give a dilution of a 1 to 100 or greater. The white pipette has the mark 11 above the bulb and the red pipette the mark 101. In addition, there is a counting chamber. This consists of a square of glass with a round hole in the center. Occupying the center of this round hole is a circular disc of glass of less diameter, so that an encircling channel is left. The square and the circle of glass are cemented to a heavy glass slide. The surfaces of each are absolutely level and highly polished. That of the circular disc is ruled into squares of varying size and is exactly 1/10 of a millimeter below the level of the surface of the surrounding glass square.

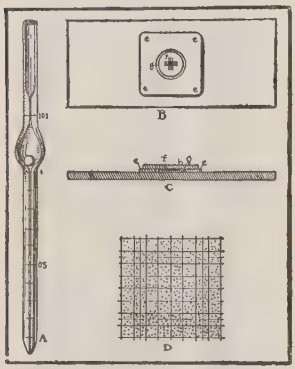

Fig. 52.—Thomas-Zeiss blood counter showing pipette, counting chamber, and ruled field. (*Greene.*)

When a polished plano-parallel cover-glass rests on the shelf, as the outer square glass is termed, there is a space left between its under surface and the ruled disc of 0.1 millimeter. The channel around the disc is termed the moat or ditch. The most desirable rulings are those of Turck and of Zappert. In these the entire ruled surface consists of nine large squares, each 1 millimeter square. These are subdivided, and in the central large square are to be found the small squares used for averaging the red cells. These small squares are 1/20 of a millimeter square and are arranged in nine groups of sixteen small squares by bordering triple-ruled lines. As the unit in blood counting is the cubic millimeter, if one counted all the white cells lying within one of the large squares (1 millimeter square), he would have only counted the cells in a layer 1/10 of the required depth, so that it would be necessary to multiply the number obtained by 10. This product, multiplied by the dilution of the blood, would give the number of white cells in a cubic millimeter of undiluted blood.

To make a red count: Having a fairly large drop of blood, apply the tip of the 101 pipette to it and, holding the pipette horizontally, carefully and slowly draw up

with suction on the rubber tube a column of blood to exactly 0.5 or 1. The variation of 1/25 of an inch from the mark would make a difference of almost 3%. If the column goes above 0.5, it can be gently tapped down on a piece of filter-paper until the 0.5 line is cut. Now insert the tip of the pipette into some diluting fluid and, revolving the pipette on its long axis while filling it by suction, you continue until the mark 101 is reached. A variation of 1/25 of an inch at this mark would only give an error of about 1/30 of 1%. After mixing thoroughly by shaking for one or two minutes, the fluid in the pipette below the bulb is expelled (this, of course, is only diluting fluid). A drop of the diluted blood of a size just sufficient to cover the disc when the cover-glass is adjusted, is then deposited on the disc and the cover-glass applied by a sort of sliding movement, best obtained by using a forceps in one hand assisted by the thumb and index-finger of the other.

Among diluting fluids Toisson's is probably the best:

Sodium chloride,	1 gram
Sodium sulphate,	8 grams
Glycerine,	30 c.c.
Distilled water,	160 c.c.

Dissolve the sodium chloride and the sodium sulphate in the glycerine water and add sufficient methyl or gentian violet to give a rich violet tint.

A 2 1/2% solution of potassium bichromate makes a very satisfactory diluting fluid in the counting of red cells.

A salt solution of about 1% strength, tinged with about 1 drop of a saturated alcoholic solution of gentian violet to about 50 c.c., is a good substitute, or the salt solution alone will answer when no white count is to be made at the same time as the red one.

It is important to work quickly in adjusting the cover-glass, or there will be cells settling in the center of the drop from a greater depth than the one which the apposition of the cover-glass makes (1/10 millimeter deep).

A good preparation should show:

1. Presence of Newton's rings.
2. Absence of air bubbles.
3. Entire surface of ruled disc covered.
4. Equal distribution of cells.

Before counting, about five minutes should be allowed for the settling of the cells.

It will be remembered that the small squares are 1/20 millimeter square. The depth of fluid from upper surface of shelf to lower surface of cover-glass is 1/10 mm. Hence each space embraced by the small square and the depth of fluid is 1/4000 of the unit used in estimating number of corpuscles in blood, or 1 cubic millimeter ($1/20 \times 1/20 \times 1/10 = 1/4000$). Count 100 of the small squares (this enables one to use decimals). There are nine squares between triple-ruled lines, each containing sixteen small squares. Count the number of corpuscles in the sixteen small squares contained in upper left-hand triple-ruled square. Put down this count. Next

count corpuscles in the adjoining sixteen squares. Put down this count. Then in third sixteen squares. Put down the number. Now move down to next row of three triple-ruled squares. Count the number of corpuscles in each of the three sixteen-square spaces and set down the numbers for addition. We have now counted ninety-six small squares (6×16). Count at any place four additional small squares and add number of blood-cells contained therein to those in the ninety-six small squares already counted. Divide the sum by 100 or simply point off two decimals. This gives the average for each small square. Multiply this by the dilution and then (as the small square is only 1/4000 cu. mm.) by 4000. This will give the number of corpuscles in 1 cubic millimeter. Example: 100 small squares contained 655 red cells. Pointing off, 6.55 equals average number of red cells per small square. Multiply by dilution (200) and then by 4000 (the small square is 4000 times smaller than the unit: 1 cu. mm.)—6.55×200=1310×4000=5,240,000.

At least 100 small squares, and preferably 200 should be counted. If the blood appears normal, one may simply count the number of red cells in five of the sixteen small square spaces (eighty small squares). Having added the numbers and multiplying by 10,000, you obtain the number of cells in 1 cubic millimeter. (Eighty small squares is 1/50 of the unit of 1 cu. mm., or 4000 small squares. The blood dilution being 1 to 200, we have 50×200×number of cells in eighty small squares.)

In counting, count corpuscles lying on the lines above and to the right. Do not count those lying on lines below and to the left.

In the small squares count only corpuscles lying in the space or cutting the upper line. This prevents counting the same cell twice.

To Count White Cells.—Draw up the fluid in the white pipette to the mark 0.5. Then, still holding the pipette as near the horizontal as possible, because the column of blood tends to fall down in the larger bore, draw up by suction a diluting fluid which will disintegrate the red cells without injuring the whites. The best fluid is 0.3% of glacial acetic acid in water. This makes the white cells stand out as highly refractile bodies. Some prefer to tinge the fluid with gentian violet. The 0.5 mark is preferred because it takes a very large drop of blood to fill the tube up to the 1 mark and if there is much of a leukocytosis a 1 to 10 dilution is not sufficient. In leukæmic blood it is better to use the red pipette with the 0.3% acetic acid solution.

The blood having been drawn up to 0.5, we have a dilution of 1 to 20. Making a preparation, exactly as was done in the case of the red count, we count all of the white cells in one of the large squares (1 sq. mm.). The cross ruling greatly facilitates this. Note the number. Then count a second and a third large square. Strike an average for the large squares counted and multiply this by 10, as the depth of the fluid gives

a content equal to only 1/10 of a cubic millimeter. Then multiply by the dilution. Example: First large square 50; second large square 70; third large square 60. Average 60. Then $60 \times 10 \times 20 = 12{,}000$, the number of leukocytes in 1 cubic millimeter of blood. The count may be made with a low power (2/3-inch objective) as the leukocytes stand out like pearls. It is better, however, to use a higher power, so that pieces of foreign material may be recognized and not enumerated as white cells.

When it is desired to make a white count with the same preparation as is used for the red one, especially if the ruling is of the old style (only central ruling and not in nine large squares as with Zappert and Türck), it is advisable to make use of the method of counting by fields. With a Leitz No. 4 ocular and a No. 6 objective, with a tube length of 12c millimeters, it will be observed that the field so obtained has a diameter of eight small squares. Now, remembering that the area of a circle equals the square of the radius multiplied by π, or 3.1416, we have the following calculation: The diameter being eight small squares, the radius would be four small squares. Squaring the radius, we have sixteen. This multiplied by 3.1416 gives us fifty. This means that every field, with the microscope adjusted as stated, contains fifty of the small squares, or 1/80 of the unit of one cubic millimeter of the diluted blood.

By keeping a single red cell in view while moving the mechanical stage from right to left or from above downward, we know that a new field of fifty small squares is brought into view when the circumference of the field cuts this individual cell. Example: As 2000 small squares would ordinarily be a sufficient number to count for a white count, this would require us to count the number of leukocytes in forty of the designated microscopic fields (this, of course, is only one-half the unit, hence we should multiply by 2). Counted forty fields and noted fifty white cells. $50 \times 2 = 100 \times 200$ (the dilution in red pipette) $= 20{,}000$. Consequently 20,000 would represent the number of leukocytes in one cubic millimeter of the blood examined.

After making a blood count, the hæmacytometer slide should be cleaned with soap and water and then rubbed dry, preferably with an old piece of linen. As the accuracy of the counting chamber depends upon the integrity of the cement, any reagent such as alcohol, xylol, etc., and, in particular, heat, will ruin the instrument. The pipettes should be cleaned by inserting the ends into the tube from a vacuum pump, as a Chapman pump. First draw water or 1% sod. carbonate solution through the pipette, then alcohol, then ether, and finally allow air to pass through to dry the interior. If the interior is stained, use 1% HCl in alcohol. If a vacuum pump is not at hand, a bicycle pump or suction by mouth will answer.

Preparations for the Study of Fresh Blood.

Many authorities prefer a fresh-blood specimen to a stained dried smear in the study of parasites of the blood. In malaria in particular there is so much information as to species to be obtained from a fresh specimen that the employment of this method should never be neglected.

While waiting for the film to stain one has five or six minutes which could not be better spent then in examining the fresh specimen which only requires a moment to make.

Manson's Method.—Have a perfectly clean cover-glass and slide. Touch the apex of the exuding drop of blood with the cover-glass and drop it on the center of the slide. The blood flows out in a film which exhibits an "empty zone" in the center. Surrounding this we have the "zone of scattered corpuscles," next the "single layer zone" and the "zone of rouleaux" at the periphery. It is well to ring the preparation with vaseline. When desiring to demonstrate the flagellated bodies in malaria, it is well to breathe on the cover-glass just prior to touching the drop of blood.

The Method of Ross is very easy of application and gives most satisfactory preparations. Take a perfectly clean slide, and make a vaseline ring or square of the size of the cover-glass. Then, having taken up the blood on the cover-glass, drop it so that its margin rests on the vaseline ring. Gently pressing down the cover-glass on the vaseline makes beautiful preparations which keep for a very long time. If it is desired to study the action of stains on living cells, this method is also applicable. A very practical way to do this is to tinge 0.85% salt solution containing 1% sodium citrate (the same as is used in opsonic work) with methylene azur, gentian violet, or methyl green. With a Wright bulb pipette, take up one part of blood, then one part of tinted salt solution. Mix them quickly on a slide and then deposit a small drop of the mixture in the center of the vaseline ring and immediately apply a cover-glass and press down the margins as before. This method will be found of great practical value.

A Method for Making Differential Leukocyte Count in same Preparation as for White Count.

Employ the same technic as in making the ordinary white count but using as a diluting fluid a 2% formalin solution to which has been added one drop of Giemsa's stain for each c.c. just before making the blood examination.

The best results are obtained when the mixing in the pipette bulb is done immediately after taking up the blood and diluent. Recently I have found it necessary to add enough N/1 NaOH to the commercial formalin to bring it to +1. Of this I use 1 1/2% in a 1/2% glycerine solution instead of water.

The usual technic in making the hæmocytometer preparation is employed-using a Türck ruling. Count the leukocytes in the three upper or lower 1 sq. mm. squares, divide by 3 to obtain an average per sq. mm., multiply by 10 for the content of a cubic millimeter and then by 20 for the dilution. (Blood to 0.5; diluent to 11.) This can be done mentally and requires no calculation on paper. Having counted the leukocytes, again go over the same portion of the ruled surface and count the polymorphonuclears and estimate the percentage of these to the total leukocytes. The majority of disrupted cells in a dry-stained preparation are transitionals hence the percentage of polymorphonuclears by this method is lower.

It is unnecessary in such a count to have an assistant; of course, in making a complete differential count it is preferable to have some one tabulate or laboriously to do this one's self.

The red cells are practically diaphanous and not disintegrated as when acetic acid is used as a diluent, consequently it is easy to make out the particular red cell as to size, etc., containing a malarial parasite.

The best results are obtained with a 1/6-in objective. Higher powers are of course impracticable by reason of the thickness of the cover-glass of the hæmocytometer.

The following are the appearances of the various leukocytes.

Eosinophiles.—In these the bilobed nucleus stains rather faintly and the color is greenish blue. The eosinophile granules show easily as coarse, brickdust-colored particles.

Polymorphonuclears.—The nucleus stains a deep, rich, pure violet but less intense than that of the small lymphocyte. The shape of the nucleus is typically three or four lobed but even when of the horseshoe shape of a transitional nucleus is easily recognizable by the intensity of the violet staining. That which makes the polymorphonuclears very easy of differentiation is the distinctness of the cell outlines produced by the fine yellowish granulations in the cytoplasm.

Small Lymphocytes.—The nucleus is perfectly round and stains a deep violet. It is almost impossible to make out any cytoplasmic fringe.

Large Lymphocytes.—The nucleus here is round and of a lighter violet than that of the small lymphocyte. The cytoplasm is blue, nongranular, and sharply defined from the nucleus.

Large Mononuclears.—These show a washed-out, slate-colored nucleus which blends with the gray slate-blue staining of the cytoplasm so that there is an indefiniteness of outline in the more or less irregularly contoured nucleus.

Transitionals.—These show the same characteristics as the large mononuclears, but with a more faintly stained and more indented nucleus. The large mononuclears and transitionals stand out as slate-colored cells. When very much degenerated these cells have a greenish hue.

Mast Cells.—The granulations show as a rich maroon or reddish-violet color.

The young ring forms of malaria show as violet-blue areas in the red cells. When half-grown or approaching the merocyte stage, the containing red cell takes on a faint pink coloration, thereby differentiating it from the noninfected red cells. At the same time the parasite is extruded and has the appearance of a violet-blue body projecting from the margin of the red cell. It is as if a blue body were budding from a pink one.

It is an easy matter with this method to count the number of trypanosomes or malarial crescents in a cubic millimeter of blood.

Preparation and Staining of Dried Films.

When preparations are desired for a differential count, Ehrlich's method of making films is to be preferred, as the different types of leukocytes are more evenly distributed. In making smears by spreading,

there is a tendency for the polymorphonuclears to be concentrated at the margin while lymphocytes remain in the central part of the film.

In Ehrlich's method we have perfectly clean dry cover-slips. Take up a small drop of blood without touching the surface of the ear or finger. Drop this cover-glass immediately on a second one and as soon as the blood runs out in a film, draw the two cover-slips apart in a plane parallel to the cover-glasses. Slide them apart. Ehrlich uses forceps to hold the cover-glasses to avoid moisture from the fingers.

Of the various methods of spreading films on slides there is none

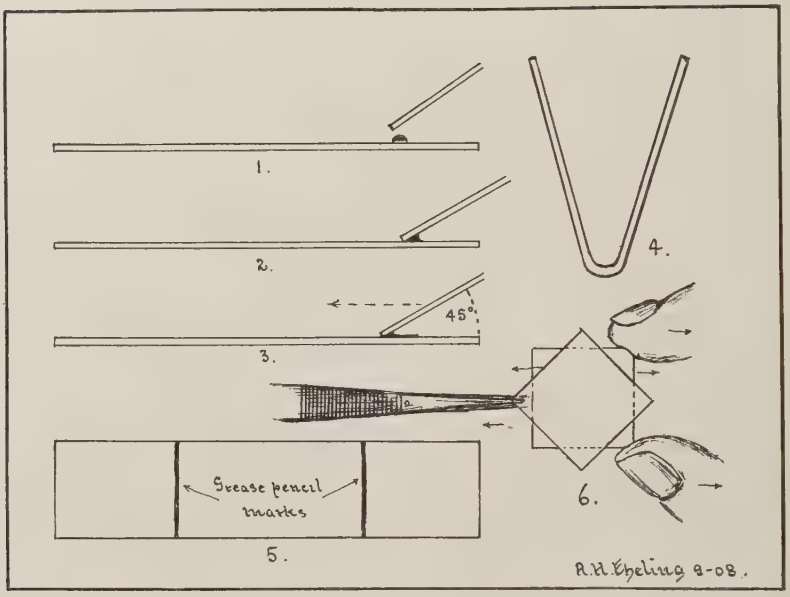

FIG. 53.—Blood technic. 1, 2, 3, Method for making blood smear on slide; 4, U tube for resting slides while staining; 5, slide showing grease pencil marking, marking prevents stain from overflowing; 6, method for drawing apart cover-glasses in making blood smear.

equal to that described by Daniels. In this the drop of blood is drawn along and not pushed along. The films are even, can be made of any desired thickness by changing the angle of the drawing slide, and there is little liability of crushing pathological cells. Take a small drop of blood on the end of a clean slide. Touch a second slide about 1/2 inch from end with the drop and as soon as the blood runs out along the line of the slide end, slide it at an angle of 45° to the other end of the

horizontal slide. The blood is pulled or drawn behind the advancing edge of the advancing slide. An angle less than 45° makes a thinner film; one greater, a thicker film.

Instead of a slide a square cover-glass may be used and if the edge be smooth it makes a more satisfactory spreader than the slide. Many workers prefer the Ross thick-film method in examining for malaria. In this about one-half of a drop of blood is smeared out over a surface about equal to that of a square cover-glass and allowed to dry. It is then flooded with a 0.1% aqueous solution of eosin for about 15 minutes. The preparation is then gently washed with water and then treated with a polychrome methylene-blue solution. After a few seconds this is carefully washed off and the preparation dried and examined.

Instead of the Daniels method some prefer to take up the drop of blood on the slide on which the smear is to be made, about 1/2 inch from the end. Then apply the spreader slide and so soon as the drop runs along the end of the spreader slide proceed as above described.

Of the various methods of making smears by means of cigarette paper, rubber tissue, needles, etc., the best seems to be to take a piece of capillary glass tubing and use this instead of a needle in making the film. There is one advantage about the strip of cigarette paper touched to the drop of blood and drawn out along the slide or cover-glass, and that is that it is almost impossible not to make a working preparation by this method.

In the making of smears the chief points are to make the smears as soon after taking the blood as possible and to have slides and cover-glasses scrupulously clean. It is well to flame all slides and cover-glasses which are to be used for blood-work. This is the best method of getting rid of grease.

Fixation of Film.—In Wright's, Leishman's, and other similar stains the methyl-alcohol solvent causes the fixation. In staining with Giemsa's stain, Ehrlich's tri-acid, hæmotoxylin and eosin, Smith's formol fuchsin, and with thionin, separate fixation is necessary. For Giemsa and thionin, either absolute alcohol (ten to fifteen minutes), or methyl alcohol (two to five minutes) answer well.

Formalin vapor, for five to ten seconds, is also used for fixation. For Ehrlich's tri-acid, hæmotoxylin and eosin and formol fuchsin, heat gives the best results. The best method is to place the films in an oven provided with a thermometer. Raise the temperature of the oven to 135° C. and then remove the burner. After the oven has cooled, take out the fixed slides or slips.

Some prefer to place a crystal of urea on the slide, then hold it over the flame until the urea melts. This shows that a temperature between 130° and 135° C. has been reached.

One of the handiest methods is to drop a few drops of 95% alcohol on the slide or cover-glass. Allow this to flow over the entire surface; then get rid of the excess of alcohol by touching the edge to a piece of filter-paper for a second or two. Then light the remaining alcohol film from the flame and allow the burning alcohol to burn itself out. A chemical fixation which gives good fixation for hæmatoxylin

and tri-acid stains (not equal to heat) is a modification of Zenker's fluid (Whitney). To Muller's fluid, which is potassium bichromate 2 grams, sodium sulphate 1 gram, and water 100 c.c., add 5 grams of bichloride of mercury and 5 c.c. of nitric acid (C. P.). Fixation is obtained in five seconds.

When using corrosive sublimate fixation one should after thorough washing in water treat the film with Gram's iodine solution for about two minutes and then wash with 70% alcohol until the yellow color of the film disappears. Methyl alcohol for two minutes is satisfactory.

Staining Blood-films.—As separate staining with eosin and methylene blue rarely gives good preparations and as the modifications of the Romanowsky stain recommended are easy to make and employ, and give much greater information, the separate method of staining is not recommended. The most satisfactory single stain is thionin.

Rees' Thionin Solution.—Take of thionin 1.5 gram, alcohol 10 c.c., aqueous solution of carbolic acid (5%) 100 c.c. Keep this as a stock solution. It should be at least two weeks old before using. For use, filter off 5 c.c. and make up to 20 c.c. with water.

1. Fix films (a) by heat, (b) by alcohol and ether, or (c) preferably by 1% formalin in 95% alcohol for one minute.

2. Stain for from ten to twenty minutes. Wash and mount. Malarial parasites are stained purplish; nuclei of leukocytes, blue; red cells, faint greenish-blue.

Ehrlich's Triacid or Triple Stain.—There are required:

1. Sat. aq. sol. orange G. (Dissolve 3 grams in 50 c.c. water.)
2. Sat. aq. sol. acid fuchsin. (Dissolve 10 grams in 50 c.c. water.)
3. Sat. aq. sol. methyl green. (Dissolve 10 grams in 50 c.c. water.)

These three solutions may be kept as stock solutions. They keep well in the dark. To make the stain, add 9 c.c. of No. 2 (acid fuchsin) to 18 c.c. of No. 1 (orange G.). After they are mixed thoroughly, add 20 c.c. of No. 3 (methyl green). Then after the first 3 ingredients are well mixed, add 5 c.c. of glycerin. Mix, then add 15 c.c. of alcohol; again mix, and finally add 30 c.c. of distilled water. Keep the mixed stain about one week before using. The best fixatives are heat and Whitneys' modified Zenker. To use, stain films from two to five minutes; then wash and mount. The triacid stain is a good tissue stain. The objections to the tri-acid stain are that it does not stain malarial parasites or mast cells and that failure to obtain good results is of frequent occurrence.

Wright's Method.—The stain is made by adding 1 gram of methylene blue (Grubler) to 100 c.c. of a 1/2% solution of sodium bicarbonate in water. This mixture is heated for 1 hour in an Arnold sterilizer. The flask containing the alkaline methylene-blue solution should be of such size and shape that the depth of the fluid does not exceed 2 1/2 inches. When cool, add to the methylene-blue solution 500 c.c. of a 1 to 1000 eosin solution (yellow eosin, water soluble). Add the eosin solution slowly, stirring constantly until the blue color is lost and the mixture becomes purple with a yellow metallic luster on the surface, and there is formed a finely granular black precipitate. Collect this precipitate on a filter-paper and when thoroughly dry (dry in the incubator at 38° C.) dissolve 0.3 gram in 100 c.c. of pure

methyl alcohol (acetone free). Wright lately has recommended using 0.1 in 60 c.c. methyl alcohol. This constitutes the stock solution. For use filter off 20 c.c. and add to the filtrate 5 c.c. of methyl alcohol.

A modification by Balch is very satisfactory. In this method instead of polychroming the methylene blue with sodium bicarbonate and heat, the method of Borrel is used. Dissolve 1 gram of methylene blue in 100 c.c. of distilled water. Next dissolve 0.5 gram of silver nitrate in 50 c.c. of distilled water. To the silver solution add a 2 to 5% caustic soda solution until the silver oxide is completely precipitated. Wash the precipitated silver oxide several times with distilled water. This is best accomplished by pouring the wash-water on the heavy black precipitate in the flask, agitating, then decanting and again pouring on water. After removing all excess of alkali by repeated washings, add the methylene-blue solution to the precipitated silver oxide in the flask. Allow to stand about ten days, occasionally shaking until a purplish color develops. The process may be hastened in an incubator. When polychroming is complete, filter off and add to the filtrate the 1 to 1000 eosin solution and proceed exactly as with Wright's stain.

In Leishman's method the polychroming is accomplished by adding 1 gram of methylene blue to 100 c.c. of a 1/2% solution of sodium carbonate. This is kept at 65° C. for twelve hours and allowed to stand at room temperature for ten days before the eosin solution is added. The succeeding steps are as for Wright's stain.

In all Romanowsky methods distilled water should be used. If not obtainable, the best substitute is rain-water collected in the open and not from a roof.

Method of staining:

1. Make films and air dry.

2. Cover dry film preparation with the methyl-alcohol stain for one minute (to fix).

3. Add water to the stain on the cover-glass or slide, drop by drop, until a yellow metallic scum begins to form. It is advisable to add the drops of water rapidly in order to eliminate precipitates on the stained film. Practically, we may add 1 drop of water for every drop of stain used.

4. Wash thoroughly in water until the film has a pinkish tint.

5. Dry with filter-paper and mount.

Red cells are stained orange to pink; nuclei, shades of violet; eosinophile granules, red; neutrophile granules, yellow to lilac; blood platelets, purplish; malarial parasites, blue; chromatin, metallic-red to rose-pink

Giemsa's Modification of the Romanowsky Method.—This is one of the most perfect of the modifications. The objection is that greater time in staining films is required than with the Wright or Leishman method and the stain is very expensive.

Take of Azur II eosin 0.3 gram. Azur II 0.08 gram.

Dissolve this amount of dry powder in 25 c.c. of glycerine at 60° C. Then add 25 c.c. of methyl-alcohol at the same temperature. Allow the glycerine methyl-alcohol solution to stand overnight and then filter. This is the stock stain. To use: Dilute 1 c.c. with 10 to 15 c.c. of dis-

tilled water. If 1 to 1000 potassium carbonate solution is used instead of water it stains more deeply.

The alkaline diluent is used to obtain the course stippling in malignant tertian (Maurer's clefts). Having fixed the smear with methyl alcohol for one to five minutes, pour on the diluted stain, and after fifteen to thirty minutes wash off and continue washing with distilled water until the film has a slight pink tinge. For Treponema pallidum stain from two to twelve hours.

While the Romanowsky methods are more satisfactory for differential counts and for the demonstration of the malarial parasites, and especially for differentiating species, yet by reason of the liability to deterioration in the tropics of methylene blue the hæmatoxylin methods may be preferable. Many workers in blood-work and cytodiagnosis prefer the hæmatoxylin.

1. Fix the film either by heat with methyl alcohol for two minuetes or with Whitney's fixative. Heat is to be preferred.
2. Stain with Meyer's hemalum or Delafield's hæmatoxylin for from five to fifteen minutes according to the stain. Frequently three minutes will be found sufficient. To make the hemalum, dissolve 0.5 gram of hæmatin in 25 c.c. of 95% alcohol. Next dissolve 25 grams of ammonia alum in 500 c.c. of distilled water. Mix the two solutions and allow to ripen for a few days. The stain should be satisfactory in two or three days.
   To make Delafield's hæmatoxylin, dissolve 1 gram of hæmatoxylin crystals in 6 c.c. of 95% alcohol. Add this to 100 c.c. of saturated aqueous solution of ammonia alum. After exposure to light for a week, the color changes to a deep blue-purple. Add to this ripened stain 25 c.c. of glycerine and 25 c.c. of methyl-alcohol and, after it has stood for about two days, filter. The stain should be filtered from time to time as a sediment forms. This makes a stock solution which should be diluted 10 to 15 times with water when staining.

Mink's Modification of Unna's Hæmatoxylin.

Hæmatoxylin,	1 gram.
Alum,	8 grams.
Sulphur (sublimed),	1 gram.
Glycerine,	30 c.c
Alcohol,	50 c.c..
Water,	100 c.c.

Dissolve the hæmatoxylin in the glycerine in a mortar. Dissolve the alum in the water and add it to the glycerine hæmatoxylin in the mortar. Then add the sulphur and the alcohol. The solution ripens in about three to four days. Allow the sediment to remain in the bottom of the bottle containing the stain and filter off small quantities as needed.

3. Wash for two to five minutes in tap water to develop the hæmatoxylin color.
4. Stain either with a 1 to 1000 aqueous solution of eosin or with a 1/2 of 1%

eosin solution in 70% alcohol. The eosin staining only requires fifteen to thirty seconds.

5. Wash and examine.

Iodophilia.

This reaction is supposed to be due to the presence of glycogen, especially in the polymorphonuclears, in suppurative conditions.

It has been stated that a differentiation between the joint involvement in gonorrhœal infection and in articular rheumatism may be made from iodophilia being present in the gonococcus infection.

Make blood-smears on cover-glasses as usual, and after they dry, but without fixation, mount them in a drop of the following solution:

Iodine,	1 part.
Potassium iodide,	3 parts.
Gum arabic,	50 parts.
Water,	100 parts.

Small brown masses in the polymorphonuclears indicate a positive iodophilia.

Viscosity of the Blood.—This is estimated by observing the relative height to which blood rises in capillary tubes as compared with water, and normally varies from three to five. The higher the hæmoglobin content the greater the viscosity. Viscosity is high in arterio-sclerosis and diabetic coma, low in the anæmias of nephritis.

Coagulation Rate of Blood.—This determination is of value in connection with operations on jaundiced patients.

Wright's coagulometer is a standard instrument but is cumbersome.

A simple method of determining the rate is to take a piece of capillary glass tubing and hold it downward from the puncture to let it fill for 3 or 4 inches. Then at intervals of thirty seconds scratch with a file the capillary tubing at short distances and break off between the fingers. When coagulation has taken place a long worm-like coagulum is obtained. Normally coagulation occurs in about three to four minutes, when the temperature is that of the hand in which the tubes are conveniently held. Rudolf recommends placing the tubes in metal tube containers in a Thermos bottle at 20° C. He gives the normal coagulation rate for this temperature as 8 minutes, while at a temperature below this the period is lengthened. Age and sex do not influence the rate. Sabrazes, the originator of this method found no appreciable variation in tubes from 0.8 to 1.2 mm. diameter.

In Bürker's test you mix a drop of blood and a drop of distilled water on a slide and with a capillary tube sealed off at the end stir the mixture every half minute. So soon as fibrin threads appear you have coagulation.

Specific Gravity of the Blood.

Hammerschlag has a method for the determination of the Hb. percentage based upon the specific gravity of the blood.

In this method a mixture of benzol and chloroform is made of a specific gravity of about 1050. A medium size drop of blood is then taken up with a pipette and dropped into the mixture. If it sinks add more chloroform from a dropping bottle, if it tends to rise, more benzol. The mixture in which the drop of blood tends to remain stationary, near the top of the mixed benzol and chloroform, has the same specific gravity as that of the blood. This is determined by an accurately graduated hydrometer. The normal average specific gravity for men is 1059, for women 1056. A table, giving the Hb. percentage corresponding to the specific gravity accompanies the outfit.

To determine the necessity for intravenous infusion in cholera Rogers has recently recommended the employment of small bottles containing aqueous solution of glycerine with specific gravities varying from 1048 to 1070, increasing the specific gravity in each successive bottle by 2°.

An accurate urinometer will suffice to determine the specific gravity. Drops of blood from the cholera patient are deposited at the center of the surface of the fluid in the bottles from a capillary pipette. If the specific gravity of the blood is 1062 at least a liter of saline or sodium bicarbonate solution is needed. If 1066 at least two liters. Formerly he estimated the indications by blood pressure considering a pressure of 80 in Europeans or of 70 in natives as indicating intravenous injections.

Occult Blood.

When the presence of blood cannot be recognized by macroscopical or microscopical methods (occult blood) we must resort to spectroscopic or chemical tests. It is in connection with blood in the fæces that these tests for occult blood are chiefly called for. Before making such tests on fæces it is advisable to have the patient on a meat-free and green-vegetable-free diet for two or three days. It is chiefly in carcinoma or ulcerations of the gastro-intestinal tract that such examinations of the fæces are required.

Hæmin Crystal Test (Teichman).—Prepare a solution of 0.1 gram each of KI, KBr, and KCL in 100 c.c. of acetic acid. This is a stable solution. Mix some of the material with a few drops of the solution on a slide, apply a cover-glass and warm the material until bubbles begin to appear (gentle steaming), then examine for dark-brown crystals.

Blood in the Urine.—The most rapid method of detection is by using the microspectroscope. An ordinary hand spectroscope will answer however.

Donogany's test is very satisfactory. To 10 c.c. of urine add 1 c.c. ammonium sulphide solution and 1 c.c. of pyridin. The urine will assume a more or less deep orange color according to its blood content. The spectrum of alkaline methæmoglobin or hæmochromogen will be obtained. See illustrations under urine.

In making the guaiac or other tests it is a good plan to repeatedly filter the blood-containing urine through the filter. Then touch a spot on the moist filter with the guaiac or benzidin solution and then finally drop on this so treated spot a drop or two of hydrogen peroxide solution.

Blood in Fæces or Gastric Contents.—Take 5 grams of fæces and rub it up thoroughly in a mortar with 15 c.c. of a mixture of equal parts of alcohol, glacial acetic acid and ether. Filter through an unmoistened pleated filter paper repeatedly until only 3 to 4 c.c. remain of the filtrate. The fæces filtrate can be first tested chemically by depositing a few drops in the center of 3 or 4 circles of white filter-paper placed in a Petri dish or upon an ordinary white plate.

The moistened spot is then treated with a few drops of a freshly prepared alcoholic solution of guaiac resin (about ½ gram of guaiac resin is broken up into small fragments and shaken up in about 3 c.c. of alcohol) and finally there is dropped upon the spot a few drops of a solution of hydrogen peroxide. Waves of blue color extending out into the moistened filter-paper show a positive test for blood.

For the benzidin test pour on this fæces filtrate-moistened filter-paper a few drops of the following solution: 2 c.c. of a saturated alcoholic solution of benzidin 2 c.c. of solution of peroxide of hydrogen and two drops of glacial acetic acid. (Blue.)

If the aloin test is preferred we treat the filtrate-moistened filter-paper with a few drops of a 3% solution of aloin in 70% alcohol and then treating the spot with hydrogen peroxide solution. Brick red colour.

More reliable is the spectroscopic test. For this we take about 3 c.c. of the concentrated ether, acetic acid, alcohol fæcal filtrate and add to it 2 c.c. of pyridin. Then add not more than 2 to 3 drops of ammonium sulphide solution. (The ammonium sulphide solution should be kept in an amber-colored, glass-stoppered bottle. The solution should be freshly prepared every 10 days.) Examine the solution, contained in a small test-tube, with the spectroscope and the two absorption bands of methæmoglobin-alkaline (hæmochromogen), between D and E, show a positive blood test. Comparison should be made with fresh blood, in which the absorption band in the yellow is nearer line D (oxyhæmoglobin spectrum).

CHAPTER XIV.

NORMAL AND PATHOLOGICAL BLOOD.

In considering what may be termed normal blood, it must be borne in mind that the normal varies for men, women, and children:

	Hb.	Red cells.	Leukocytes.
Men,	90 to 110%,	5 to 5 1/2 million,	7500.
Women,	80 to 100%,	4 1/2 to 5 million,	7500.
Children,	70 to 80%,	4 1/2 to 5 million,	9000.

Color Index.

This is obtained by dividing the percentage of the hæmoglobin by the percentage of red cells, five million red cells being considered as 100%. To obtain the percentage of red cells it is only necessary to multiply the two extreme figures to the left by two. Thus if a count showed the presence of 1,700,000 red cells, the percentage would be 34 ($17 \times 2 = 34$). If the Hb. percentage in this case were 50; then the color index would be $50 \div 34$, or 1.4.

In normal blood the color index is, approximately, 1.

In anæmias we have three types of color index: 1. The pernicious anæmia type, which is above 1. Here we have a greater reduction in red cells than we have of the hæmoglobin content of each cell. 2. The normal type, when both red cells and hæmoglobin are proportionally decreased, as in anæmia following hæmorrhage. 3. The chlorotic type. Here there is a great decrease in hæmoglobin percentage, but only a moderate decrease in the number of red cells. Hence the color index is only a fraction of 1. For example, in a case of chlorosis we have 40% of hæmoglobin and 90% of red cells, $40 \div 90 = 0.4$.

Red Cells.

In considering the corpuscular richness of a specimen of blood, it must be remembered that this does not necessarily bear any relation to the quantity of blood in the body. Thus, a more or less bloodless-

looking individual, the total quantity of whose blood is greatly reduced, may, notwithstanding, give a normal red count. In examining a specimen of peripheral blood we get a qualitative, not a quantitative result.

Normally, we have an increase in red cells in those living at high altitudes. An altitude of two thousand feet may increase the red count about one million, and a height of six thousand feet about two million. Profuse sweats and diarrhœas also increase the red count. Pathologically, in chronic polycythemia with cyanosis and splenic enlargement, we have a red count of about ten million. In cyanosis from heart disease, etc., and in Addison's disease there is also an increase in red cells.

The normal red cell or erythrocyte measures about 7.5μ in diameter. It is non-nucleated and normally stains with acid dyes, taking the pink of eosin or the orange of orange G. If larger, 10 to 20μ, it is called a macrocyte; if smaller, 3 to 6μ, a microcyte.

Anisocytosis is a term applied to a condition where marked variation in size of the red cells occurs.

Macrocytes are rather indicative of severe forms of anæmia, the microcytes, of less grave types. When the red cell is distorted in shape, it is called a poikilocyte. Care must be exercised that distorted shapes are not due to faulty technic. Crenation and vacuolation of red cells are marked in poorly prepared specimens.

In addition to variation in size and shape, we also have pathological variation in staining affinities.

Polychromatophilia.—This shows itself by red cells taking a brownish to a dirty blue tint, as is frequently seen in immature red cells, especially nucleated ones.

Granular basophilic degeneration (also termed punctate basophilia and stippling) refers to the presence of blue dots in the pink background of stained red cells. It is found in many severe anæmias, as pernicious anæmia, the leukæmias, malarial cachexia, etc. It is very characteristic of lead poisoning.

The nucleated red cell, while normal for the marrow, is always pathological for the blood of the peripheral circulation. Normoblasts have the diameter of a normal red cell. The nucleus is round and stains intensely with basic dyes, often appearing almost black. Another characteristic is that it frequently appears as does the setting in a ring. Some give the term microblast to smaller nucleated forms. In normoblasts the red cell proper stains normally. The megaloblasts not only have a greater diameter than the normoblast, but the nucleus is poor in chromatin, stains less intensely and is less distinctly outlined. In-

stead of being round, the nucleus is irregular and may be trefoil in shape. The cytoplasm surrounding the nucleus shows polychromatophilia. This contrasted with the pure blue of the lymphocytes should differentiate. Normoblasts are found in secondary anæmias, and especially in myelogenous leukæmia. Megaloblasts are peculiarly characteristic of pernicious anæmia. Enormous megaloblasts are sometimes termed gigantoblasts.

In aplastic anæmia (a severe type of pernicious anæmia), in contrast to ordinary pernicious anæmia, nucleated reds are very rarely found. There is also very little poikilocytosis, and the color index is about normal. It is a rare, rapidly fatal anæmia, particularly of young women.

It does not show remissions, runs a rapid course, and is attended with a marked increase of lymphocytes. The bone marrow of the femur is pinkish yellow and homogeneous.

The term leukanæmia has been employed to describe conditions which partake of the characteristics of pernicious anæmia and leukæmia.

White Cells.

Owing to the conflicting views as to origin, nature, and functions of the various leukocytes, their classification is in a state of confusion. As regards the appearance of the cells, this of course varies as the stain used, and it requires considerable experience for a single individual to be able to positively recognize the difference between a lymphocyte and a large mononuclear when one specimen is stained with a Romanowsky stain, another with Ehrlich's triacid, and a third with hæmatoxylin and eosin. This, of course, is intensified when different persons adhere to the method of staining which they prefer and are at a loss to appreciate differences which are brought out by some other stain used by some other person. Even with the same stain used with different specimens of blood we find the staining characteristics of various leukocytes imperceptibly merging the one into the other, so that at times it is impossible for one, even with his own standard of differentiation, to be sure whether he is dealing with a lymphocyte or a large mononuclear. The difficulty is even greater when we deal with Türck's irritation forms and with myelocytes.

Without going into the various granule stainings so thoroughly brough out by Ehrlich, we shall immediately take up the question of a practical classification for use in making a differential count. As the Romanowsky method of staining (Wright, Leishman, or Giemsa) gives

us information not yielded by either hæmatoxylin and eosin or the triacid, the points of differentiation to be referred to in that which follows is with blood so stained.

In considering the staining affinities of different parts of the leukocytes, it is convenient to divide such into basic ones, acid ones, and those which may be said to be on the border line between these—the so-called neutrophilic affinities.

With Wright's stain we have the eosinophile or oxyphile affinity of the granules of eosinophiles for acid dyes, in this case eosin. The nuclei and basophile granules have affinities in greater or less degree for basic stains (the blue and the violet shading resulting from methylene blue as modified by polychroming). With the granules in the cytoplasm of the polymorphonuclears and neutrophilic myelocytes, and to a less extent in the transitional, we have a staining which merges into a yellowish-red on the one extreme and into a lilac on the other. As a standard, neutrophilic granules should be a mean of these extremes.

Not only by reason of the authority of Ehrlich, but because such a division gives all variations, which can then be combined by one preferring a simpler classification, it would seem proper to divide the normal leukocytes into:

1. **Small Lymphocytes.**—These are small round cells about the size of a red corpuscle with a large centrally placed, deeply violet staining nucleus and a narrow zone of cytoplasm. This cytoplasm may not be more than a mere crescentic fringe. This is the type of lymphocyte which makes up the greater proportion of the leukocytes in chronic lymphatic leukæmia. At times these cells seem to be composed of nucleus alone.

2. **Large Lymphocytes.**—These are of the same type as small lymphocytes, but possessing more cytoplasm. The nucleus, while round and taking a fairly deep rich violet stain, does not stain so deeply as the nucleus of the small lymphocytes. The cytoplasm is a clear, translucent, pure blue. It may contain pinkish granules known as azur granules, but these are of rather large size and do not mar the glass-like appearance. They are from 9 to 15μ in diameter and are common in children. In the acute lymphatic leukæmias they at times predominate.

3. **Large Mononuclears.**—These are large round or oval cells with a nucleus which has lost the richness of violet staining of the lymphocyte nucleus. The nucleus is furthermore frequently irregular in outline or may show the commencing indentation of the transitional nucleus.

There is not that sharp distinction between nucleus and cytoplasm that exists in the lymphocytes. The cytoplasm of the large mononuclear gives the impression of opacity, as if it were frosted glass instead of clear glass. The neutrophile mottling which begins to appear causes a disappearance of the pure blue character of the cytoplasm of the lymphocyte. It is principally by the washed-out staining of the nucleus and the opaque lilac of the cytoplasm that we differentiate them from the lymphocytes. They greatly resemble Türck's irritation forms or plasma cells and may be confused with myelocytes.

4. **Transitionals.**—These appear as but a later stage in the decay of the large mononuclears; the nucleus is more indented, frequently horseshoe-shaped, and has a washed-out violet shade of less intensity than that of the large mononuclears. These are the cells so often disrupted in smears.

These four kinds of cells are frequently referred to as the lymphocyte series, and although many authorities consider that the small lymphocyte represents a more mature cell than the others of this class, yet it is thought by others that the age of the cell increases as we go from small lymphocytes to large lymphocytes, thence to the large mononuclear; and then in the transitional we have the decrepit stage which precedes dissolution. The old view that the transitional was the precursor of the polymorphonuclear has few advocates at the present time.

While it is convenient to consider these hyaline cells as representing different stages in development, yet from a standpoint of immunity this is untenable. The large mononuclears and transitionals are the cells in which we find certain animal cells and pigment phagocytized, as is the case in malaria. These cells are the macrophages of Metchnikoff and are probably derived from the bone marrow.

The lymphocytes take origin from the lymphoid tissue, and very probably the large lymphocyte is a younger, more immature cell than the small lymphocyte.

Ehrlich and Naegeli regard the large mononuclears as of myeloid origin while Pappenheim considers them to belong to the group of lymphocytes.

A normal percentage of large mononuclears and transitionals combined should not exceed about 4%.

In addition to the series of leukocytes just considered we have present normally in the blood three types of granular cells distinguished according to the staining affinity of their granules. These are:

1. **Polymorphonuclear Leukocytes.**—This cell normally constitutes the greater proportion of the leukocytes. It is an amœboid, actively phagocytic cell, about 10 or 12μ in diameter, and is the microphage of Metchnikoff. Bacteria are actively phagocytized by this cell, and it is the cell concerned in determining the opsonic power of blood to various bacteria. It has fine lilac granules which are termed neutrophilic (epsilon granules). The single nucleus is rich in chromatin and is lobose like the kernel of an English walnut; frequently it resembles the letter z. These cells are derived from the neutrophilic myelocytes of the bone marrow. It is in these cells that the glycogen, or iodophil granules, appear in certain suppurative conditions.

A great deal of interest has been aroused in the so-called Arneth index, especially in connection with prognosis in tuberculosis and various pyogenic infections. The basis of the test is that polymorphonuclears showing only one or two nuclear nodes are considered immature while those having three, four or five nuclear nodes possess greater phagocytic power.

A normal distribution is as follows:

Class I.	Class II.	Class III.	Class IV.	Class V.
6%	35%	42%	16%	1%

To obtain the Arneth index add to the sum of the polymorphonuclear percentages of cells containing one and two nodes one-half of the percentage of those having three nodes. In the above we have as the normal Arneth index 62.

In an advanced case of tuberculosis we might have an index of 79, obtained as follows:

Class I.	Class II.	Class III.	Class IV.	Class V.
20%	45%	28%	6%	1%

2. **Eosinophile Leukocytes.**—These are very striking cells with coarse granules staining brilliantly pink, the eosinophile, oxyphile, or acidophile granules (alpha granules of Ehrlich). The cells are a little larger than the polymorphonuclears. The normal eosinophile is to be distinguished from the eosinophilic myelocyte by its possessing two distinct lobes in the nucleus. At times we find three nuclei. The nucleus of the myelocyte is round. The eosinophile is the cell so frequently increased in infections by intestinal animal parasites.

3. **Mast Cells.**—These also have coarse granules, but they stain a deep violet blue. Hence they are basophile granules (gamma granules). In fresh blood these granules do not show up very well, thus they can be distinguished from the highly refractile granules of the eosinophile.

The trilobed nucleus stains less intensely than the granules. As a rule, the mast cell is about the size of a polymorphonuclear.

In a differential count of normal blood we find about the following percentages.

Polymorphonuclears,	65 to 70%,	about	5000 per c. mm.
Small lymphocytes,	20 to 25%,	about	1500 per c. mm.
Large lymphocytes,	5 to 10%,	about	500 per c. mm.
Large mononuclears,	1 to 2%,	about	100 per c. mm.
Transitionals,	2 to 4%,	about	200 per c. mm.
Eosinophiles,	1 to 2%,	about	100 per c. mm.
Mast cells,	1/4 to 1/2%,	about	25 per c. mm.

The leukocytes which are found in the peripheral circulation only in pathological conditions are:

1. **Neutrophilic Myelocytes.**—The common type is a large cell with a large centrally placed, feebly staining nucleus. This may be recognized by the difficulty of distinguishing the nucleus from the cytoplasm, there being no sharp line separating these parts of the cell. They imperceptibly merge into one another. They differ from a large mononuclear in that the cytoplasm is distinctly dotted with neutrophile granules and that we cannot make out a distinct line of separation of a slightly irregular or indented nucleus from the surrounding slightly neutrophilic cytoplasm. Cornil has described a very large myelocyte with eccentrically placed nucleus and neutrophilic granules.

Myelocytes are at times found with both basophilic and neutrophilic granules, and may rarely be seen to have all three kinds of granules on a single myelocyte, acidophile, basophile, and neutrophile.

2. **Eosinophilic Myelocytes.**—These can be distinguished from normal eosinophiles by their possessing a single round nucleus, not bilobed. These myelocytes may be as large as a normal eosinophile, but frequently are no larger than a red cell.

The neutrophile myelocyte is characteristic of spleno-myelogenous leukæmia, the eosinophile one of myelogenic leukæmia. The occurrence of an occasional myelocyte is frequently noted in conditions having a leukocytosis. In diphtheria their presence in numbers is of bad prognostic import. Myelocytes are of diagnostic importance in metastases of malignant tumors.

3. **The Irritation Cell of Turck, or Plasma Cell.**—This cell has a faintly staining, eccentrically placed nucleus, and a dark opaque blue, frequently vacuolated, cytoplasm. They are usually recorded as large

mononuclears. Turck supposed them to appear in the circulation as the result of bone-marrow irritation.

4. **Myeloblasts.**—These cells are found in myeloid leukæmia and though often mistaken for lymphocytes or large mononuclears they are of marrow origin. The nucleus stains more intensely than that of the large mononuclear and the cytoplasm is more deeply blue stained than that of the large lymphocyte. They also contain three or four nucleoli.

Pyronin methyl-green staining is best for demonstrating the nuclei.

5. **Pathological Large Lymphocytes.**—These are as a rule much larger than normal large lymphocytes and show poorer staining of both nucleus and cytoplasm. The nuclei often show the appearance of division into two or more lobes, thus showing the characteristics of Rieder cells. They may be confused with large mononuclears but are considered to be derived from the germinal centers of various lymphoid tissues. They are found in leukæmic and pseudo-leukæmic conditions.

Blood Platelets.

These are normally present in blood in the number of about 350,000 per cubic millimeter. They disintegrate very quickly after the blood is withdrawn. Wright has demonstrated that they are pinched-off projections of giant cells of the bone marrow. They consist only of protoplasm, no nuclear material. They do not contain hæmoglobin. In conditions where giant cells are less abundant, as in pernicious anæmia, the blood platelets are less abundant. In myelogenous leukæmia they are very abundant. They vary in size from 2 to 5μ according as a larger or smaller pseudopod of a giant cell has been broken off. Stained with Wright's stain, they are more purplish than blue and show thread-like projections. They are often mistaken for the protozoal causes of various diseases. Especially are they confused with malarial parasites when lying on a red cell. The blood plate has no brick-red chromatic material; it is purplish rather than blue, and has no pigment grains. It is advisable to compare these isolated blood plates with the larger or smaller aggregations scattered about the smears. In this way their true character is apparent. In addition to blood platelets, which in fresh blood can only be observed when a fixative is used, we have other confusing bodies.

The hæmokonia of Muller are small, highly refractile bodies showing active oscillatory movement. They are supposed to be cast-off granules of eosinophiles or other leukocytes, or possibly derived from nuclei. As this blood dust or hæmokonia is found in a marked degree in lipæmia it may be that the particles are fat. It is interesting that this lipæmia is absent after the taking of large quantities of fat in cases with serious pancreatic trouble. The serum of a normal individual is rather turbid after slight indulgence in butter. Pinched-off fragments of red cells may also appear as possible protozoal bodies.

Leukopenia.

This is a term used to designate a reduction in the normal number of leukocytes. A leukocyte count of 5000 would represent a slight leukopenia; one of 2000, a marked leukopenia. In the later stages of typhoid, and in acute miliary tuberculosis, we expect a moderate leukopenia.

The leukopenia of typhoid is moderate and is often preceded in the first few days by a moderate neutrophile leukocytosis. Later on we have a decided increase in the lymphocytes. A marked diminution or absence of eosinophiles is so characteristic that any increase in eosinophilic percentage negatives a diagnosis of typhoid.

Paratyphoid gives a similar blood picture.

Chronic alcoholism and chronic arsenic poisoning cause a reduction in the number of the white cells. Pernicious anæmia shows a marked leukopenia, as is also the case with Banti's disease. Two tropical diseases, kala-azar and dengue, show a marked leukopenia, the counts often being below 2500. During the apyrexial period of malaria we may have a white count of 5000.

It has recently been claimed that a leukopenia with a coincident marked reduction in the lymphocytes is characteristic of measles and that this occurs several days before the Koplik spots appear.

Kocher notes that in exophthalmic goiter the leukocyte count is considerably diminished and that the polymorphonuclears are not much more than one-half the usual percentage while the percentage of the lymphocytes is almost double the normal.

X-ray treatment tends to destroy leukocytes in the exposed region, especially polymorphonuclears. The small lymphocytes are least affected.

Eosinophilia.

Where the eosinophiles are increased to 5%, we have a moderate eosinophilia. In some cases of infection with intestinal parasites, especially hook-worms, but also from other parasites, as round and whip-worms, we may have an eosinophilia of 30 to 50%. In Guam, among the natives, it is difficult to find an eosinophile count under 15%. The eosinophilia tends to disappear when the anæmia becomes very severe.

The eosinophilia of trichinosis is best known, and a combination of this blood finding with fever and marked pains of mucles, would justify the excision of a piece of muscle for examination for encysted embryos.

In true asthma eosinophilia is marked, and its absence is of value in indicating other causes for the condition. Certain skin diseases, especially pemphigus, show eosinophilia.

Eczema and psoriasis are not apt to give more than 3 or 4% eosinophiles. A rather high degree of eosinophilia is found in mycosis fungoides.

Scabies also gives an eosinophilia.

The proportion of eosinophiles in the blood of children is greater than in that of adults.

Increase of both eosinophiles and mast cells is found in myelogenous leukæmia.

Leukocytosis.

It is to an increase in the polymorphonuclears that this term is usually applied, the term lymphocytosis or eosinophilia being employed where white cells of eosinophile or lymphocyte nature are increased. We have physiological leukocytosis in the latter weeks of pregnancy, also in the new-born, and in connection with digestion.

Pathological Leukocytosis.— Pneumonia. In this disease we have a leukocytosis of 20,000 to 30,000 or higher. The eosinophiles are almost absent. A normal leukocyte count in pneumonia makes a prognosis unfavorable.

The leukocyte count drops about the time of the crisis, and with the reappearance of eosinophiles is a favorable sign. A moderate leukocytosis occurs in carcinoma and sarcoma.

Septic processes. The leukocyte count is of great value, especially when we obtain a leukocytosis with 80 to 90% of polymorphonuclears, as in appendicitis, cholecystitis, or other suppurative conditions.

According to Cabot, leukocytosis varies in infections as follows:

1. Severe infection—good resistance; early, marked and persistent leukocytosis.
2. Slight infection—slight resistance; leukocytosis present, but not marked.
3. In fulminating infections we may have no increase in whites, but a higher percentage of polymorphonuclears.
4. Slight infection and good resistance may not be productive of leukocytosis.

It is in connection with the question of operation in appendicitis or similar conditions that the matter of a leukocyte count is of prime importance. If there be a leukocytosis but with less than 75% of

polymorphonuclears it indicates an infection of little virulence or a walled-off process with an exacerbation. It is difficult to form an opinion when the polymorphonuclears are under 80%. Leukocytosis with polymorphonuclear percentage of 85 to 90 indicates immediate operation; percentages over 90 point to peritonitis and if with such percentages of polymorphonuclears there is absence of leukocytosis the prognosis is grave.

Spirochæta fevers, as relapsing fever, may give a leukocytosis of from 25,000 to 50,000.

Smallpox, especially at time of pustulation, plague, scarlet fever, and liver abscess give a leukocytosis of from 12,000 to 15,000.

Smallpox often shows a very large percentage of very characteristic large mononuclears.

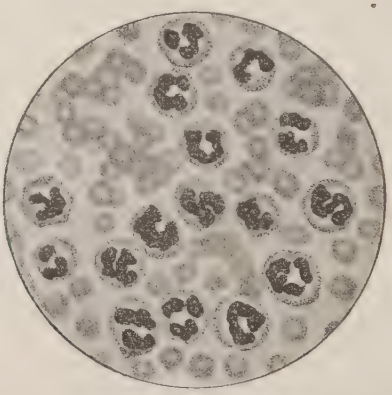

FIG. 54.—Leukocytosis (40,000); sixteen polymorphonuclears in field. (*Cabot.*)

The leukopenia and lymphocyte increase in measles are important points in differentiating it from scarlatina.

With meningitis counts of 25,000 are not unusual, in abscess of the brain the white count rarely exceeds 15,000.

Poliomyelitis and polioencephalitis give a slight leukocytosis during the febrile accession.

Erysipelas and epidemic cerebrospinal meningitis also give a leukocytosis of from 15,000 to 20,000. In malignant diseases we sometimes have a moderate leukocytosis. Rogers states that in liver abscess, with a leukocytosis of 15,000 to 20,000, we have only about 75 to 77% of polymorphonuclears—there being also a moderate increase in the percentage of large mononuclears.

Drugs such as antipyrin may give a leukocytosis. The leukocyte increase of pilocarpine is rather a lymphocytosis.

Lymphocytosis.

Of course, the disease in which we have the most marked lymphocytosis is lymphatic leukæmia.

The lymphocytosis of typhoid fever has been taken up under leukopenia.

Whooping-cough may give a lymphocytosis of 20,000 to 30,000.

Young children have normally an excessive proportion of lymphocytes. This is apt to be particularly marked in hereditary syphilis. Enlarged tonsils may give rise to a lymphocytosis of 10,000 to 15,000, when more than 50% of the white cells will be lymphocytes. Rickets and scurvy give a lymphocytosis.

Varicella and mumps may also give an increase in the percentage of lymphocytes.

Malta fever is a disease which may show quite a mononuclear increase.

Diseases in which there is a Normal Leukocyte Count.

Uncomplicated tuberculosis, influenza, Malta fever, measles, trypanosomiasis, malaria, syphilis, and chlorosis. In malaria we have a leukocytosis at the time of the rigor, while during the apyrexial period there is a moderate leukopenia. In malaria we have a marked increase in the percentage of the large mononuclears and transitionals. These may form from 25% to 35% of the leukocytes. When bearing particles of pigment they are known as melaniferous leukocytes—macrophages which have ingested malarial material. In dengue, at the time of the terminal rash, we may have as great a percentage of large mononuclears. In this disease, however, we have a great diminution of polymorphonuclears from the start (25 to 40%). Instead of a large mononuclear we have at the onset a lymphocytic increase. There is an increase of large mononuclears in trypanosomiasis.

The white count is about normal in uncinariasis (Ashford's average was 7800). Some have reported a leukopenia in severe cases.

While eosinophilia is the most marked feature in hook-worm disease yet in very severe cases it may be absent.

The Primary Anæmias.

Chlorosis.—In chlorosis it is the reduction of hæmoglobin with the slight numerical variation from normal of the red cells that makes for a diagnosis. The color index is very low. There is nothing abnormal

about the leukocytes. Microcytes may be present, and very occasionally a normoblast. Macrocytes and megaloblasts are always absent. Blood of chlorotics is very pale and very fluid and coagulates rapidly, hence frequency of thrombosis.

Spleen, liver, and lymph glands as a rule normal.

Simple Primary Anæmia.—This condition is not recognized by many authors, but is a convenient term under which to group anæmias which are neither chlorosis nor pernicious anæmia and for which no assignable cause can be designated. It is a secondary anæmia without a cause. In it color index is about normal, there is no change in the leukocytes and cases go on to recovery.

Pernicious Anæmia.—In pernicious anæmia we obtain a very fluid, but normally colored drop of blood upon puncture. The yellow

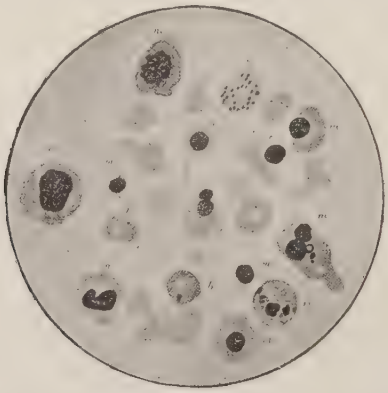

FIG. 55.—Pernicious anæmia. *M.m*, Megaloblasts; *n*, normoblast; *s*, stippling (punctate basophilia). (Cabot.)

marrow of the long bones is transformed into a soft, bright red lymphoid tissue, smears from which show great numbers of megaloblasts. Areas of fatty degeneration are characteristic, especially the tiger-lily spots in the heart muscle. Iron-containing pigment (hemosiderin) is found in the liver, spleen, and kidneys. Areas of degeneration in the spinal cord may account for nervous symptoms. The red cells frequently fall below 2,000,000 with patients going about. Cases have been reported with counts under 200,000. The color index is high. Megaloblasts are the most characteristic qualitative change in the red cells. Megaloblastic crises may at certain times show enormous numbers of megaloblasts. Cases often present remissions in which no megaloblasts can be found. In such cases the presence of many macrocytes

should prevent an examiner's reporting against a pernicious anæmia previously diagnosed.

Poikilocytosis, polychromatophilia, and stippling are also features of the disease. Normoblasts are far less frequent than megaloblasts and there is usually a moderate lymphocytosis. Myelocytes may be present, but their precursors, the myeloblasts, are probably more frequently met with.

Cases of pernicious anæmia show remissions during which the patient is apparently on the road to recovery. Such improvements are only temporary. The remissions may last from two months to possibly three or four years. Especially in the anæmia of Dibothriocephalus latus do we have a picture of pernicious anæmia. It is supposed to be due to a toxin present in the heads of these tape-worms.

Blood changes more or less like those of pernicious anæmia have at times been noted in children with tuberculosis of bovine nature. The human strain of T.B. does not seem to produce such changes.

An acute disease showing a rapidly developing anæmia of the pernicious anæmia type is verruga peruana in which the bone marrow seems especially involved.

Secondary Anæmias.

These are the anæmias which can be definitely traced to some disease not of the hæmopoietic system.

There are two main groups—those following hæmorrhage and those secondary to various diseases. If the hæmorrhage is sudden and great, the resulting condition is one of oligochromæmia—chlorotic in type. Normoblasts are usually found after the third day.

The low Hb. percentage is apt to continue for several weeks. There is also an increase in the percentage of polymorphonuclears.

It is a question whether prolonged operation or those requiring narcosis are justified where the reduction in Hb. is under 40%. (According to Miculicz, 30% is the minimum).

Where the loss of blood is gradual, as in gastric cancer or severe hæmorrhoids the picture may more nearly approach that of pernicious anæmia. Secondary anæmias usually show a moderate leukocytosis. In chronic nephritis and prolonged suppurative conditions normoblasts and macrocytes are rare—moderate poikilocytosis with the presence of many microcytes being the rule.

In fatal anæmia from chronic acetanilide poisoning high color index, macrocytes and megaloblasts have been noted.

In some secondary anæmias, as in syphilis, carcinoma, and tuberculosis, we have a chlorotic color index (chloro-anæmias).

In secondary anæmias polychromatophilia, poikilocytosis, and punctate basophilia (stippling)may be present. This latter is very marked

in lead poisoning, but in certain cases of malarial cachexia it may be equally prominent. The only form of nucleated red cell seen is the normoblast, in very small numbers, or it may not be present.

Megaloblasts are practically never seen, except in some of the very severe parasitic anæmias, as the broad Russian tape-worm infection. The red cells generally number between 2,000,000 and 4,000,000, thus differentiating chlorosis. The leukocytes are frequently increased to 15,000. In the anæmia of splenic anæmia there is a marked leukopenia. In anæmias from malignant tumors the color index is usually of the chlorotic type—the hæmoglobin content of the red cells being more affected than the number. Normoblasts are usually present, and this finding may differentiate gastric cancer from ulcer. In bone marrow metastases megaloblasts may be expected. Myelocytes and so-called tumor cells (large cells with faintly-staining vacuolated nuclei and but little cytoplasm) may also be found. As a rule, there is a moderate leukocytosis in malignant disease. Eosinophiles may be largely increased in sarcoma.

The Leukæmias.

It is in the leukæmias that we have the greatest increase in the number of white cells. These cases show more or less anæmia, but we may have cases of myelogenous leukæmia showing 250,000 leukocytes per cubic millimeter without particular change in the red cells. The more marked the red-cell change the more severe the condition.

There are two well-defined types of leukæmia, the lymphatic and the splenomyelogenous. It must be borne in mind, however, that while a greater change in the lymphatic glands may produce the lymphatic type, yet even in such cases we expect to find alteration in bone marrow and spleen; that is, there is a general involvement of the hæmopoietic system in all leukæmias, the activity being most marked in spleen and bone marrow in certain cases and in lymphatic glands in others.

Myelogenous leukæmia is a very rare disease, about five times as rare as pernicious anæmia. Lymphoid leukæmia is still more rare.

Splenomyelogenous Leukæmia (myeloid leukæmia).—The differentiation of the blood picture of this disease from leukocytosis does not depend on the number of leukocytes, but on the presence and large proportion of myelocytes. We expect both neutrophilic and eosinophilic myelocytes in myeloid leukæmia—the proportion of these varies, but, as a rule, the neutrophilic one is the common one. The blood in advanced cases is milky and shows a most marked buffy coat. The marrow is largely replaced by a yellow pyoid material. The spleen may weigh 10 pounds.

The leukocyte count is on the average from 200,000 to 500,000. Cases are reported of more than 1,000,000 white cells. The neutrophilic myelocytes make up about 30 to 40% of these and, about equal in number, are found the polymorphonuclears, while the percentage of the lymphocytes is decreased (2 to 5%) and normal eosinophiles, eosinophilic myelocytes, and large mononuclears make up the remaining percentages. We usually have great numbers of normoblasts. Megaloblasts may be rarely found. The red count is usually about 2,500,000 and the color index low.

Lymphatic Leukæmia.—In this we have glandular enlargements, but not such large masses as in Hodgkin's disease. The red cells are usually reduced about one-half and the color index is a little below normal. Normoblasts are rarely found. Myelocytes, as a rule, are

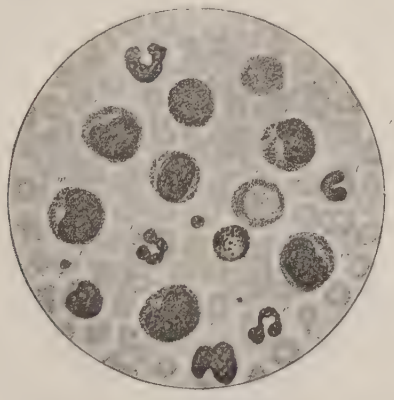

FIG. 56.—Myelogenous leukæmia. *m*, Myelocyte; *p*, polymorphonuclear; *b*, mast cell; *n*, normoblast. (*Cabot.*)

absent, but may amount to 5% of the leukocytes. The predominating leukocyte (75 to 98%) is the small lymphocyte. In acute lymphatic leukæmia the large lymphocytes predominate.

These however are pathological and differ from the large lymphocyte in not having azur granules and the nucleus stains poorly and is often indented. The leukocyte count is never so great as in myeloid leukæmia, rarely exceeding 125,000.

Pseudoleukæmia.—Hodgkin's disease is usually considered as a disease with marked glandular enlargements, but with a negative blood picture, or at any rate only a moderate leukocytosis with a relative increase of lymphocytes.

The red cells are usually above 3,000,000. It has been considered that an increased percentage of transitionals (10 to 15%), should a leukopenia coexist, is characteristic.

Undoubtedly the view that so-called lymphosarcomata, lymphatic leukæmia, and Hodgkin's disease merge into one another and that they represent a malignant cell formation in the hæmopoietic system is the conservative one to take.

A certain proportion of cases of Hodgkin's disease, however, show endothelial proliferation and a chronic fibroid change.

In **Kundrat's lymphosarcoma** we have a neutrophile leukocytosis and a diminution of the lymphocytes. The spleen and liver are rarely involved.

Another condition with swelling of the lymphatic glands, which do not however fuse, is the so-called granulomatosis.

In this we have a polymorphonuclear leukocytosis of from 20,000 to 50,000 with

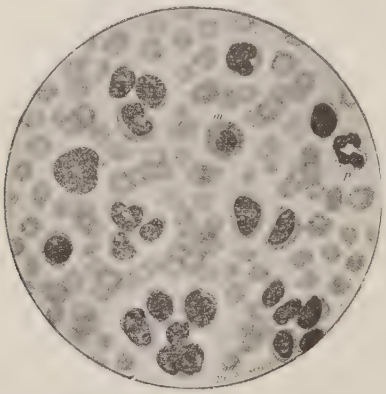

FIG. 57.—Lymphatic leukæmia. *p*, polymorphonuclear; *m*, megaloblast; *e*, eosinophile. Twenty-one lymphocytes in this field. (*Cabot.*)

an increase in the percentage of eosinophiles. The lymphocytes are absolutely and relatively decreased. In granulomatosis there is no tendency to hæmorrhage.

Splenomegaly.—The best known anæmia associated with splenic enlargement is Banti's disease.

Banti's disease also has a very low color index and leukopenia. In this the primary affection is of the spleen which becomes greatly enlarged. The accompanying cirrhosis of the liver with its symptoms of ascites, etc., differentiate it. Splenectomy often cures the disease. The leukopenia is one showing not only a diminution of polymorphonuclear percentage but of cells of the lymphocyte type as well.

There is a considerable increase in the large mononuclear percentage. Nucleated reds and myelocytes are invariably absent. It must be remembered that

we have a group of cases showing splenomegaly which are syphilitic in origin and which as a rule give a positive Wassermann. Clinically or hæmatologically they resemble true Banti's disease but pathologically the spleen shows a fibrosis instead of the marked increase in lymphatic tissue characteristic of Banti's disease.

In the tropical splenomegaly or **kala azar** we have a marked leukopenia with a marked reduction in the percentage of polymorphonuclears. The Gaucher type of splenic anæmia does not show as pronounced and early an anæmia as in Banti's type.

Certain conditions which partly resemble myelogenous leukæmia and partly pernicious anæmia are designated **leukanæmia.** Some consider this to belong to the group of diseases in which the multiple myeloma is placed.

In **splenomegalic polycythæmia** we have a red count of from 9 to 10 millions. The Hb. percentage may be 200. There is also a leukocytosis up to 50,000. Patients are cyanosed and have a very large spleen.

Splenic anæmia of infancy usually occurs between the ages of twelve and twenty-four months. The spleen is notably enlarged and in many cases the liver is equally so. The red cells are not greatly diminished in number, two and one-half to three millions being usual findings. Nucleated reds are abundant. While a leukocytosis of 30,000 to 50,000 is often present it is markedly less than that of splenomyelogenous leukæmia and the increase in white cells is more of those of lymphocyte type.

The color index is very low.

Another splenomegaly of children, clinically resembling kala azar, is caused by Leishmania infantum.

NOTES ON BLOOD WORK.

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PART III.

ANIMAL PARASITOLOGY.

CHAPTER XV.

GENERAL CONSIDERATIONS OF CLASSIFICATION AND METHODS.

Animals that are in all respects alike we term a Species. Of course the male and female of a species may be very unlike, but as a result of mating they produce young having characteristics similar to the parents. Now, if, as in the case of the mosquitoes causing yellow fever, we find some with straight silvery lines and others uniformly showing crescentic silvery bands about thorax, yet resembling each other closely in the respect of being dark, brilliantly marked mosquitoes, we should consider them as being separate species with a certain relationship to which the term Genus is applied.

The term "genus" is of wider application than the word "species." Thus animals which agree in the main characteristics of size, proportion of parts, and general structure are placed in the same genus.

In naming a species we always first write the name of the genus which has a Greek or Latin name, commencing with a capital, and follow with the specific term, which latter commences with a small letter. Thus we designate the dark silver-marked mosquitoes as belonging to the genus Stegomyia; those showing the characteristics of curved silver bands and two central parallel lines (lyre pattern) on dorsal surface of thorax we designate as Stegomyia calopus; the species with only the straight silver lines we call Stegomyia scutellaris.

If the specific name is a modern patronymic we add i in the case of a man or æ for a woman to the exact and complete name of the person.

Again, certain genera show resemblances which enable us to make broader groupings to which we apply the term Subfamily. Thus the genus Stegomyia and the genus Culex have the similar characteristics of palpi in the female being shorter than the straight proboscis; we therefore classify all species of Stegomyia

and all species of Culex under the designation Culicinæ. The name of a subfamily ends in "inæ." Now, again, certain insects are different from others in having scales on the wings. We find that not only do the Culicinæ have such characteristics, but the same is observed with the Anophelinæ and other similar scale-wing insects. All of these we term a Family and we speak of the Culicidæ, meaning the family of mosquitoes. The name of a family ends in "idæ." Many families are not subdivided into subfamilies, but are directly separated into genera. Again, a genus may have only a single species.

At times a family may be raised to superfamily rank—the subfamilies then becoming families. Thus the families Ixodidæ and Argasidæ belong to the superfamily Ixodoidea. The termination for a superfamily is oidea.

When there are a number of families agreeing closely in some striking characteristic, we group them together into an Order; thus, the family of mosquitoes closely resembling many other families of insects in possessing a pair of well-developed wings are grouped in the order Diptera; all of which resemble certain other animals in the possession of a distinct head, thorax and abdomen with three pairs of legs projecting from the thorax. This collection of animals we call a Class; thus, we speak of the class Insecta. It will be observed that the insects have no internal skeleton, but instead a chitinous cuticle, the exoskeleton. Spiders, ticks, etc., resemble them in this respect, and we now apply to all such animals the wider designation, Branch or Phylum Arthropoda.

Inasmuch as the animal kingdom is divided into the branches Protozoa, Porifera, Cœlenterata, Echinodermata, Vermes, Arthropoda, Mollusca and Chordata, we see that the branch is the largest grouping we employ. To descend in the scale we have belonging to the branch, the classes; to the class, the orders; to the order, the families; to the family, the subfamilies; to the subfamily, the genera; to the genus, the species. Occasionally a species is further divided into subspecies.

By a type species we understand the species of a genus always referred to as representing the genus.

While other species of a genus may for good reason be transferred to another genus the type species is permanently in the genus. Many favor alliteration for type species, as Heterophyes heterophyes. When a species is transferred to a new genus the specific name goes with it.

The male animal is designated by the sign of Mars (♂), the female by that of Venus (♀).

There are certain terms employed in animal parasitology which it is necessary to understand. Among these we shall refer to the following:

1. **True Parasitism.**—By this is understood the condition where the parasite does harm to the host, deriving all the benefit of the association. A good example of this would be the hookworm infecting man or animals.

2. **Mutualism.**—In such an association there is mutual benefit to each party of the association. An instance of this would be the presence of colon bacilli in the intestines. The bacillus is furnished a suitable habitat and in return protects its host against strictly pathogenic bacteria.

Another example would be the oyster crab found inside the oyster shell.

3. **Commensalism.**—Here there is benefit to the parasite, but no injury to the host. An example of this kind would be furnished in the case of the Trichomonas vaginalis which lives in the vaginal mucus, but so far as known, does no injury to the host.

If the Entamœba coli be nonpathogenic this would be another example.

4. **Nomenclature.**—When the thousands of different species, genera, etc., of animals is considered, it will be readily perceived that, unless some system existed for their designation, indescribable confusion would prevail. To avoid this, the International Code, based on the rules of Linnæus (tenth edition of Systema naturæ, 1758, is basis of binary zoological nomenclature), requires Latin or Latinized names.

In printed matter the zoological name should be in italics, that of the family in Roman type. The name of the author of a specific name is written immediately after the name without punctuation and may be followed by the year of publication set off by a comma, thus: *Ascaris lumbricoides* Linnæus, 1758. Should the name of the author appear in parentheses it indicates that he proposed the specific name but placed the species in another genus than that in which it now appears, and the name of the author responsible for placing the species in the present genus may be written after the name of the original author of the species; for example, *Davainea madagascariensis* (Davaine, 1869) Blanchard, 1891, tells us that Davaine proposed the specific name *madagascariensis* in 1869 but placed it in some other genus and that Blanchard in 1891 transferred it to the genus *Davainea*. There are certain rules governing the naming of animals. Of these, the law of priority provides that the oldest published name, under the code, of any genus or species is its proper zoological name. The history of the naming of the organism of syphilis illustrates this well. Schaudinn gave this organism in 1905 the name of Spirochæta pallida. Ehrenburg, in 1838, had used the name Spirochæta for animals of a different character, so that this designation of the genus was not permissible under the code. Villemin, a little later, proposed the generic name Spironema. This term, however, was found to have been used in 1864 by Meek for a genus of molluscs and by Klebs in 1892 for a genus of flagellates. Consequently, being a homonym, it was not available.

(A generic name can be applied to only one animal genus and if a similar name is subsequently given another genus it is a homonym and is to be rejected.)

On December 2, 1905 Stiles and Pfender then proposed the name Microspironema, but as Schaudinn published on Oct. 26, 1905 the designation Treponema, the name Treponema pallidum had to be accepted as the proper zoological name for the organism of syphilis.

Of unusual interest is the question of the name of the old-world hookworm. Dubini, in 1843, named a nematode found by him in man Agchylostoma. By the law of priority this spelling would have been the correct one had he not stated in a footnote that the generic name was derived from two Greek words *αγχύλοσ* and *στόμα*. Having indicated the origin of the name it became subject to the rules for correct transliteration, which is Ancylostoma.

In case of larva and adult or male and female, formerly considered different animals but subsequently found to be the same, the oldest available name becomes the name of the species.

Another point is that names are not definitions, consequently the fact of lack of appropriateness of any name is no objection to its continuation. This will appeal to anyone as a wise provision, because if a different name were substituted each time a designation more descriptive or applicable was invented it would be utterly destructive to system. When it is considered that some of our parasites have approximately fifty different designations, for the most part given by medical observers, it will be appreciated how much the zoologist has aided us in trying to eliminate all but the single proper zoological name.

The objections so frequently heard among physicians in connection with adopting new names for old ones are not well founded. Wherever confusion has reigned, the establishment of order always results in temporary greater confusion. There is no doubt that the student taking up this subject a few years hence will have the satisfaction, thanks to the zoologist, of only having to burden his mind with one name for one parasite.

There is only one correct name for an animal and all other names are synonyms.

The principal cause of changes of names is that our conception of the relationships of animals changes.

5. **Terminology.**—This applies to appropriate designations for different organs, symptoms, etc., and is not subject to any rule other than that of good usage.

Thus the terms cirrus in the case of the male copulatory organ of flukes, spicule for the same in nematodes and penis in connection with insects would be instances of terminology.

6. **Pseudoparasitism.**—Where organisms enter the body accidentally and when such sojourn in the body of man plays no part in the life history of the organism we employ the term pseudoparasitism. For example: Fly larvæ swallowed by man and passed out in the fæces. We also use the terms temporary parasites (bedbug) and permanent parasites (liver fluke).

7. **Hosts.**—The animal in which a parasite undergoes its sexual life is called the definitive or final host, that in which it passes its larval existence the intermediary host. For example: Man is the intermediary host of the malarial parasite, the mosquito the definitive host. A single animal may, however, be both definitive and intermediary host; thus Trichinella may pass its larval existence in the muscles of man and its sexual life in his intestines.

8. **Heredity, Congenitalism.**—Hereditary characteristics are those which were present in the ovum or spermatozoon before fertilization; congenital ones those which occur after fertilization. South African tick fever is probably an instance of heredity, the spirochætes having been found in the ovary and ova of the female tick.

9. **Heterogenesis, Parthenogenesis.**—Offspring differs from parent, but after one or more generations there is reversion to the parent form.

Strictly speaking the term heterogony applies to reproduction when a sexual

generation alternates with a parthenogenetic one. Where a nonsexual generation, as by division or budding, alternates with a sexual one the process is called metagenesis. In parthenogenesis reproduction eggs develop without the occurrence of fertilization by spermatozoa.

In coccidiosis we have a sexual cycle (sporogony) alternating with a nonsexual one (schizogony). In the infection with Strongyloides we have a sexual cycle alternating with a parthenogenetic one. In malaria we have a sexual generation, a nonsexual one and according to Schaudinn, a parthenogenetic one, which latter accounts for malarial relapses.

CHAPTER XVI.

THE PROTOZOA.

Classification of Protozoa.

Class	Order	Genus	Species
Rhizopoda (Sarcodina) These throw out protoplasmic projections called pseudopodia.	Gymnamœba	Entamœba	E. coli
			E. histolytica
			E. tetragena
			E. buccalis
		Leydenia	L. gemmipara
Flagellata (Mastigophora) These move by means of undulating membranes or flagella.		Spirochæta	S. recurrentis
			S. vincenti
			S. duttoni
			S. carteri
			S. refringens
		Schizotrypanum	S. cruzi
		Treponema	T. pallidum
			T. pertenue
		Trypanosomà	T. gambiense
			T. rhodesiense
		Trichomonas	T. vaginalis
			T. intestinalis
		Lamblia	L. intestinalis
		Babesia	B. bigemina
		Leishmania	L. donovani
			L. tropica
			L. infantum
Infusoria (Ciliata) These have contractile vacuoles and numerous fine cilia which are shorter than flagella and have a sweeping stroke.	Heterotricha	Balantidium	B. coli
Sporozoa These have no motile organs. They live parasitically in the cells or tissues of other animals. Reproduction by spores.	Coccidiaria	Eimeria	E. stiedæ
		Isospora	I. bigemina
	Hæmosporidia	Plasmodium	P. vivax
			P. malariæ
			P. falciparum

NOTE.—Hartmann and others have grouped the Hæmosporozoa and the Hæmoflagellata in an order BINUCLEATA. The main characteristic is the possession of two differentiated nuclei, the kinetonucleus and the trophonucleus, at some developmental or transitional stage. While trypanosomes plainly show these characteristics certain others, as the malarial parasites and the leishman-donovan bodies, having been modified as the result of cell parasitism, do not do so. This grouping together of the blood flagellates and sporozoa under the name Binucleata has been considered by many protozoologists as possibly convenient but not resting on sufficient ground to cause organisms with similar life histories as Plasmodium and Coccidium to be separated and the former to be placed with the blood flagellates in a new grouping.

THE PROTOZOA.

By the term protozoa we understand a branch of animals in which a single cell is morphologically and functionally complete; it is not one of a number of cells going to make up a complex individual and dependent on such a combination as is the case with the metazoa (there is no differentiation into tissues in protozoa).

Recognizing the fact that certain protozoa have characteristics which make it impossible to draw a distinction between them and plants Haeckel has proposed the name Protista as a designation for all simple and primitive living organisms whether they be plants or animals. In such a classification we would have the kingdom of Protista as well as the animal and vegetable kingdoms. In such a grouping the bacteria would be the lower types and the fungi and protozoal organisms the higher ones.

The protozoal cells are made up of protoplasm which is divided into nucleus and cytoplasm. The cytoplasm is at times separated into an external, hyaline portion, the ectoplasm or ectosarc and an internal granular portion, the endoplasm or endosarc. The functions of the ectosarc are protective, locomotor, excretory and sensory; those of the endosarc trophic and reproductive. Protozoa may be holozoic (animal like) or holophytic (plant like), saprophytic (fungus like), or parasitic (living at the expense of some other animal or plant).

The nucleus is characterized by concentration of the so-called chromatin substance of the cell. This chromatin however is usually combined with achromatin. The usually accepted test for chromatin is the staining affinity for basic aniline dyes. This test is now known to be unsatisfactory as other substances than chromatin may stain even more intensely. When chromatin is scattered through the cytoplasm, as extranuclear aggregations, such chromatin granules are called chromidia. There are cells where the chromidia take the place of the nucleus and from which a nucleus may be formed. Chromidia may arise from nuclei and nuclei from chromidia. The nucleus is made up of a network of linin in which achromatic reticulum is contained the nuclear sap or karyolymph. As a rule an achromatic nuclear membrane, continuous with the reticulum, separates the nucleus from the cytoplasm. In addition we have a substance which is achromatic (plastin) and which is the imbedding substance for chromatin grains. These plastin chromatin combinations are called karyosomes. The nucleoli are probably pure plastin. Plastin is to be regarded as a secretion or modification of chromatin

made to serve as a matrix for the chromatin. Chromatin may be concentrated in a single mass so that the nuclear space looks like a vesicle with a central chromatin mass (vesicular nucleus) or numerous chromatin grains may be scattered through the nuclear space (granular nucleus). The centrosome, which presides over cell division, is usually located just outside the nucleus. In some protozoa however the centrosome is within the nucleus and is often seen inside of a karyosome and is then called a centriole. The centrosome may also function over kinetic activities (flagellar motion) and is then termed blepharoplast.

Certain protozoa, as trypanosomes, show a differentiation of nuclei, the larger trophonucleus governing the functions of general metabolism and the smaller kinetonucleus directing the motor activities. Infusoria have a larger macronucleus which contains vegetative chromatin and a smaller micronucleus which contains reserve reproductive chromatin.

Reproduction of protozoa may be by fission, when the nucleus and cytoplasm divide into two by simple division.

When the nuclei divide into a number of daughter nuclei, which is followed by multiple division of the cytoplasm, we have sporulation.

Instead of fission we may have sexual reproduction or conjugation (zygosis). Here the nuclei of the separate sexual individuals (gametes) are termed pronuclei and the product of their fusion a synkaryon.

Where a single cell has division of its nucleus with subsequent fusion of these daughter nuclei to form a synkaryon the process is termed autogamy.

If two similar cells conjugate the term is isogamy; if dissimilar as the macrogametes and microgametes of malaria, anisogamy.

The process of sexual union is termed syngamy and is of two kinds (1) when the two gametes fuse completely or copulation and (2) when they remain separate and only exchange nuclear material or conjugation.

The structures of protozoa concerned in movement, metabolism, etc., are termed organelles. Of the former, pseudopodia, flagella, cilia and myonemes (contractile fibrils which give support to the body cell of certain protozoa) may be given and food vacuoles and contractile vacuoles of the latter. The contractile vacuole which is probably an excretory organelle is absent in almost all parasitic protozoa. It is however present in ciliates.

Rhizopoda (Sarcodina).

In this class of protozoa the pseudopodia serve the double purpose of nutrition and locomotion. These protoplasmic extensions may be quite broad or very narrow—the lobose and the reticulose.

As a rule, the thicker the pseudopod the more rapid the movement. Some rhizopods have hard shell-like coverings which are secreted in or on the ectosarc. These skeletons have openings through which the pseudopods project. The pseudopodia may be made up only of ectoplasm or both ectoplasm and endoplasm may take part. Amœboid movement always starts in the ectoplasm. In addition to the nucleus,

which the so-called chromatin-staining methods bring out as reddish areas, we frequently observe smaller aggregations of chromatin-staining material in the cytoplasm. This extranuclear chromatin is supposed to play a part in the more intricate divisions which such protozoa undergo. Food vacuoles and contractile vacuoles are present in many rhizopods.

Entamœba coli (**Amœba coli**).—This is considered by Schaudinn to be a harmless inhabitant of the intestines and its presence in the fæces is not considered of importance.

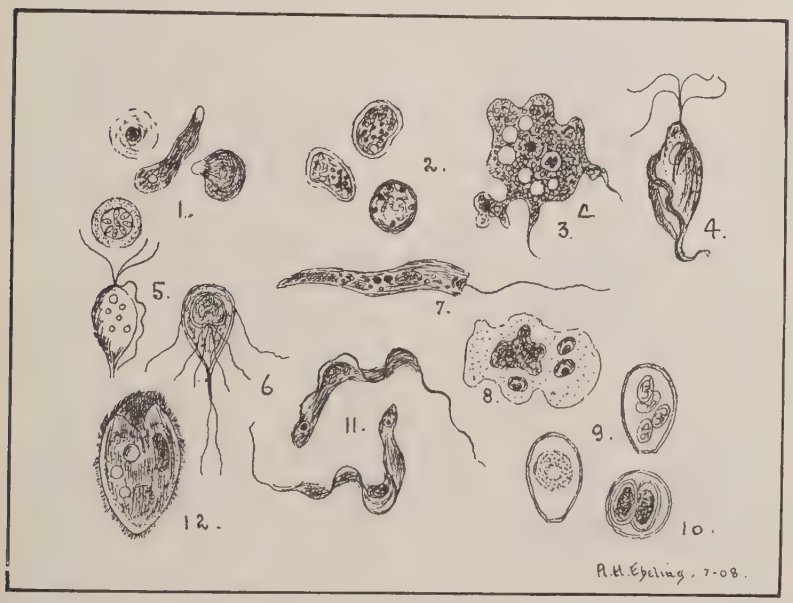


FIG. 58.—Various protozoa. 1, Entamœba coli; 2, Entamœba histolytica; 3, Leydenia gemmipara; 4, Trichomonas vaginalis; 5, Trichomonas intestinalis; 6, Lamblia intestinalis; 7, flagellated Leishmania donovani; 8, Leishmania donovani in phagocyte; 9, Eimeria stiedæ; 10, Isospora bigemina; 11, Trypanosoma gambiense; 12, Balantidium coli.

It is now recognized that amœbæ of man are not cultivable. When we obtain cultures on the various nutrient poor agar plates, formerly so much used, we find that the amœbæ belong chiefly to water amœbæ, in particular a Limax.

The only safe way in recognizing amœbæ in stools is to note amœboid movement. The encysted amœbæ, except by the experienced, can scarcely be differentiated from many vegetable cells and especially from large phagocytic cells, of probable endothelial origin. By the use of neutral red in very dilute solution the granular endoplasm will be observed to take up the brick-red stain.

A method for bringing out the nuclear features is as follows: take a loopful of 2% acetic acid and a loopful of 2% formalin. Tinge the mixture to a rose color with neutral red and then stir in a little saturated aqueous solution methyl green, using a tooth pick which has been dipped into the methyl green.

In staining with iron hæmatoxylin or better with phosphotungstic hæmatoxylin proper fixation is very important. Fix in 100 parts of sat. aq. sol. bichloride to which is added 50 c.c. absolute alcohol and 5 drops glacial acetic acid. The stain should be poured on the moist smear of fæces. The fixative should be heated to 60° C. and should only act for 10 to 20 seconds. Then place in cold sublimate alcohol for 10 minutes wash in 70% alcohol colored to a rich port wine color with iodine, then in 70% alcohol, then in water and then stain as preferred. Some like a carmine stain.

E. coli varies greatly in size (8 to 40μ). There is no well-marked distinction between a granular interior and a more compact, hyaline exterior. The nucleus is centrally situated, is distinct, and on staining with Wright's stain shows the chromatin coloration. The nucleus is rich in chromatin and with iron hæmatoxylin it shows four chromatin aggregations lining the nuclear membrane. It is sluggishly motile and is of a grayish-white color. When stained it does not show a distinction between endoplasm and ectosarc. The infecting stage is an encysted form with eight nuclei or spores.

Entamœba histolytica (**Amœba dysenteriæ**).—This is considered the pathogenic amœba. Schaudinn considered that it was by the possession of its tough, tenacious glassy, and highly refractile ectoplasm that it was able to bore its way into the submucosa of the large intestine and bring about those gelatinous-like necroses, which, by undermining, eventually result in dysenteric ulcerations.

It was also thought to be the species found in tropical liver abscess. As described by Schaudinn, it has a marked differentiation between the glassy ectoplasm and the granular endoplasm. The nucleus is indistinct, eccentric, or even peripherally situated, and stains feebly.

The movement is more active and the color more greenish-yellow than E. coli. Craig notes the characteristic staining of the E. histolytica, this being a dark blue ectoplasm encircling a lighter blue endoplasm. In dividing, there is a process of budding. These little spore-like bodies form at the periphery of the encysted amœba and are the infecting stage. Fæces should be examined as soon as possible after the stool is passed in order that one may have the best opportunity to observe movement. A particle of mucus pressed down with a cover-glass makes a satisfactory preparation. If necessary to dilute, use blood-warm salt solution—not plain water.

Amœbæ were first described by Lambl in 1859. Found by Loesch in dysenteric stools in 1875. Councilman and Lafleur in 1891 separated amœbæ into pathogenic and nonpathogenic strains.

Kartulis produced dysentery in cats by introducing dysenteric stools into the rectum. Kruse and Pasquale produced dysentery with liver abscess pus which

was bacteriologically sterile. Shiga in 1898 separated the bacillary type of dysentery from the amœbic one. Schaudinn, in 1903, stated that E. histolytica was the pathogenic amœba of man. Viereck found encysted amœbæ in dysenteric stools containing four nuclei. This amœba is now believed to be the common pathogenic amœba of man and is named E. tetragena.

Entamœba tetragena.—This amœba has a homogenous and highly refractile ectoplasm with a nucleus richer in chromatin than E. histolytica. It has a central karyosome which varies in size.

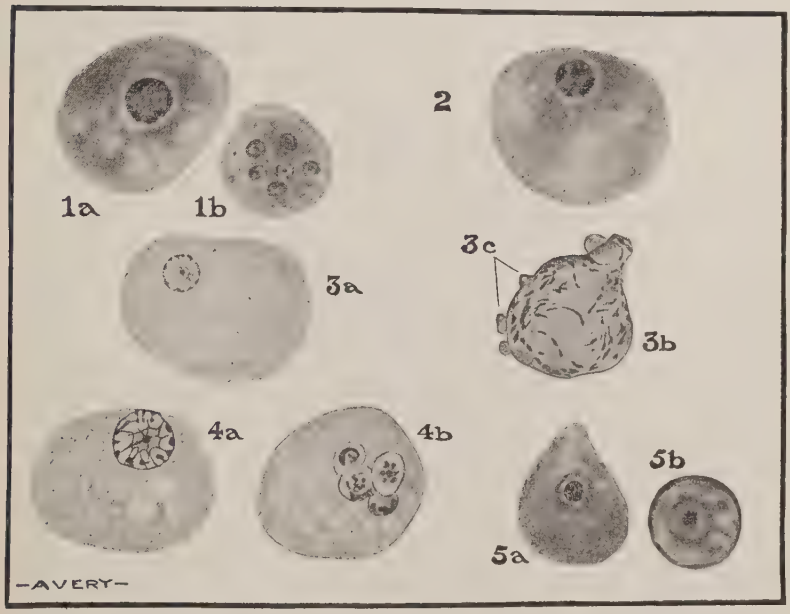


FIG. 59.—Human amœbæ showing vegetative and encysted stages. Water amœbæ for comparison. (*1a*) Entamœba coli; (*1b*) E. coli (encysted); (*2*) E. japonica; (*3a*) E. histolytica; (*3b*) E. histolytica (encysted); (*3c*) E. histolytica peripheral buds; (*4a*) E. tetragena; (*4b*) E. tetragena (encysted); (*5a*) water amœba, vegetative; (*5b*) water amœba, encysted.

In an iron hæmatoxylin preparation this karyosome shows a central spot or centriole which may fill up most of the nuclear space but in such case is surrounded by a clear zone with the karyosome ring outside. Hartmann found that some of Schaudinn's specimens were E. tetragena and the belief is now growing that the life history of a nucleus resolving into chromidia which collected at the periphery and formed the peripheral infecting spores was an error in observation on the part of Schaudinn and that the true life history of the pathogenic human amœba is that of E. tetragena. In such case E. tetragena and E. histolytica applying to the same

amœba we must drop the name E. tetragena by reason of priority of E. histolytica. Craig now takes this view.

Wenyon has recently produced dysentery in kittens by infecting them with material containing the four spores of the encysted E. tetragena. He also produced liver abscesses in one of the kittens experimentally infected with dysentery.

Entamœba buccalis.—This has an ectoplasm similar to E. histolytica, but has a centrally situated nucleus, the nucleus, however, is poor in chromatin.

Obtained from the mouths of persons with dental caries. It does not appear to have pathogenic characteristics.

Castellani has reported an intestinal amœba with an undulatory membrane. He has given it the name of E. undulans.

Leydenia gemmipara.—It is a question whether these bodies were animal parasites or simply body cells showing amœboid movement. They were found in the ascitic fluid of two cases of carcinomatosis. They varied in size from 3 to 36μ.

Flagellata (Mastigophora).

In this class of protozoa the adults have flagella for the purposes of locomotion and the obtaining of food.

Some flagellates more or less resemble rhizopods in being amœboid and in having an ectoplasm and an endoplasm. The body is frequently covered by a cuticle (periplast). Some flagellates have a definite mouth part, the cytostome, which leads to a blind œsophagus; others absorb food directly through the body wall. In addition to flagella, some flagellates possess an undulating membrane. All flagellates possess a nucleus and some have contractile vacuoles. The flagellum may arise directly from the nucleus or from a small kinetic nucleus, the blepharoplast (micronucleus or basal granule).

The most important flagellates of man are the hæmoflagellates. Among these we may include the blood spirochætes and the organism of syphilis, which have many resemblances to the spiral forms of bacteria, together with the three genera in which protozoal characteristics are marked, namely, Leishmania, Trypanosoma and Trypanoplasma. In addition we have flagellates in the intestinal canal and in the vaginal secretion. Some authors place the genus Piroplasma with the flagellates and there has been controversy concerning the nature of certain projections from these bodies. It would seem preferable, however, to consider them under the Sporozoa.

Spirochæta.

The generic term Spirochæta is applied to flagellates having a spiral shape, an undulating membrane, and no flagella. This genus is one about which there are two views: one, that the members belong to the bacteria; the other, that they are protozoa. The absence of demonstrable nucleus and blepharoplast makes them apparently vegetable in nature while the variations in thickness, the fact of transmission by an arthro-

pod, and indications of a longitudinal, rather than a transverse division, would indicate protozoal affinities.

It would seem from recent investigations that both methods occur—longitudinal division occurring when there are few organisms in the blood and transverse at the height of the infection.

Minchin has adopted the name Spiroschaudinnia, proposed by Sambon, for the parasitic blood spirochætes

S. recurrentis.—This is the organism of relapsing fever. It was formerly considered a bacterium and was termed the Spirochæta obermeieri (discovered by Obermeier in 1873).

It is present in the blood of persons suffering from the disease during the pyrexia. During the apyrexia they are not found in the peripheral circulation. At this time they are present in great numbers in the spleen where they are actively phagocytized.

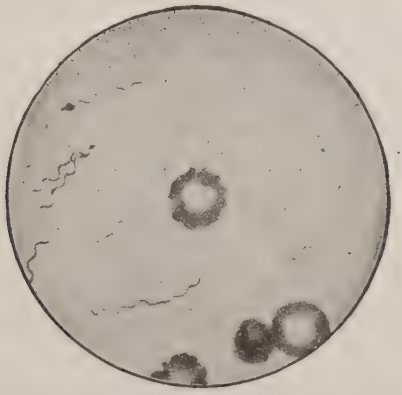


FIG. 60.—Spirochætæ of relapsing fever from blood of a man. (*Kolle and Wassermann.*)

The disease is supposed to be transmitted by bedbugs or lice. Monkeys are susceptible and, after passage of the organism through monkeys, rats can be infected.

S. duttoni.—This is the cause of South African tick fever or "tete-fever." The disease is similar to relapsing fever, but there are generally four or five febrile paroxysms with apyrexial intervals. The disease is readily transmitted to ordinary laboratory animals, especially the rat.

A certain degree of immunity is conferred by an injection with a certain spirochæte, but this does not hold for other species; thus, rats which have recovered from S. recurrentis can be infected by S. duttoni and *vice versa*. The disease is transmitted by the bite either of the adult or larval Ornithodoros moubata. Koch found spirochætes in the eggs of the ovaries of ticks which had fed on persons with the disease. It is thus an instance of hereditary transmission.

Leishman, who believes in the protozoal nature of these organisms, has observed clumps of chromatin granules in the Malpighian tubes and in the ovaries of infected ticks, which granules he considers developmental stages. Material showing such

granules but no spirochætes has brought about spirochæte infection in mice. He considers that infection probably occurs through material voided from the Malpighian tubes rather than through the medium of veneno-salivary secretions.

Other spirochætes that have been considered as pathogenic for the type of relapsing fever in India and that of America are the S. carteri and the S. novyi.

Nicolle has shown with relapsing fever of Algiers that the body louse can transmit the infection by spirochæte containing material from the crushed louse being rubbed into the wound made by the louse in biting. Eggs from an infected louse hatch out infected young lice, thus showing the hereditary transmission. It is now also considered that infection with South African relapsing fever by O. moubata occurs by the rubbing in of spirochæte containing fæces into the wound made by the bite of the tick. These, as with plague infection from the contaminated fæces of the rat flea, are instances of the contamination mode of infection. Noguchi has recently cultivated the various species of pathogenic human spirochætes by employing a method similar to that used in cultivating the organism of syphilis. He noted longitudinal division in his cultures.

S. vincenti.—This is a very delicate spiral-shaped organism which has been found in conjunction with a fusiform bacillus in a throat inflammation, usually termed Vincent's angina.

S. refringens.—This Spirochæta is frequently associated with the Treponema pallidum and is common in genital ulcerations. It is thicker, has less regular and more flattened curves and stains more readily. By "dark ground illumination" it is thicker, of a yellow tint instead of pure white, and moves in its entire length.

Treponema.

The genus Treponema has no undulating membrane and has a flagellum at each end.

Treponema pallidum (Spirochæta pallida).—This is the cause of syphilis. It is characterized by the very geometric regularity of the spirals, which are deeply cut, and in focusing up and down continue in focus (like a corkscrew). They require about thirty minutes to stain distinctly with Giemsa's stain and the attenuated ends or flagella should always be noted before reporting their presence.

Treponemata are found in the cellular areas surrounding the thickened blood-vessels and in the coats of the larger arteries. To stain them in section Levaditi's method is the best.

The India-ink method of Burri is highly recommended. Take one loopful of secretion from a chancre and deposit it on one end of a slide. Surround this drop with five loopfuls of distilled water and five loopfuls of Günther and Wagner's ink. Mix and make a smear as for blood. When dry examine with the oil immersion objective and the treponemata will be found to stand out as white spirals against a dark background. Treponemata often appear as if bent in the middle.

Harrison prefers collargol to India-ink. One part of collargol is put in a bottle with 19 parts of water and well shaken. This shaking is repeated. One loopful

of the suspected serum and one loopful of the collargol suspension are mixed and smeared out and examined as for the India-ink method.

T. pallidum has been cultivated anaerobically in horse serum by Schereschewsky. The cultures contained other organisms. Muhlens, by growing anaerobically on horse-serum agar (1 to 3), claims to have obtained pure cultures. Animal inoculations with this material were negative, however.

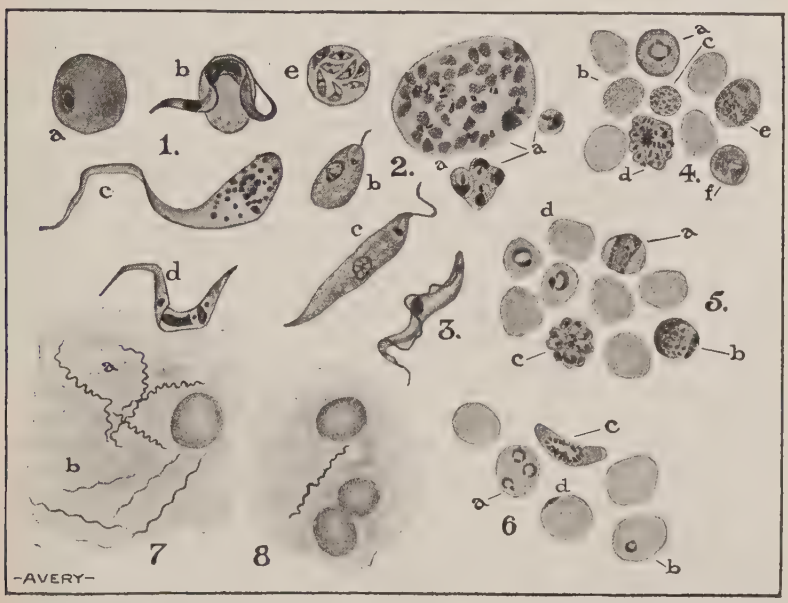


FIG. 61.—Binucleata, (Hæmoflagellata and Hæmosporozoa). 1. Schizotrypanum cruzi; (*a*) Merozoite just entering r.b.c.; (*b*) fully developed trypanosome form in blood; (*c*) form found in intestine Conorhinus; (*d*) form in salivary gland of Conorhinus; (*e*) merocyte from the schizogenous cycle in lungs. 2. Leishmania donovani; (*a*) Parasites from spleen smear, free and packed in phagocytic cell; (*b*) and (*c*) flagellate forms from cultures. 3. Trypanosoma gambiense. 4. Plasmodium vivax; (*a*) young schizont; (*b*) uninfected red cell; (*c*) red cell, punctate basophilia; (*d*) merocyte; (*e*) macrogamete; (*f*) adult schizont. 5. Plasmodium malariæ; (*a*) half-grown schizont showing equatorial band; (*b*) macrogamete; (*c*) merocyte; (*d*) young schizonts. 6. Plasmodium falciparum; (*a*) red cell showing multiple infection; (*b*) young ring form; (*c*) crescent; (*d*) young schizont on periphery of r.b.c. 7. (*a*) Treponema pallidum; (*b*) Spirochæta refringens. 8. Treponema pertenue.

Noguchi has cultivated T. pallidum under strict anaerobic conditions in a medium of ascitic fluid containing a piece of fresh sterile tissue, preferably placenta. The growth is faintly hazy and does not have an offensive odor. Spirochæta microdentium shows similar morphology but the cultures have a foul odor. Sp. macrodentium is similar culturally but differs morphologically.

When cultures of T. pallidum, grown for one or more weeks in ascitic fluid agar

and ascitic fluid are ground in a mortar, heated to 60° C. for one hour then, with the final addition of 1% trikresol, we have an emulsion called "luetin." This extract produces an allergic reaction on the skin of certain syphilitics (Luetin reaction). To carry out the test luetin is introduced intradermally at the insertion of the left deltoid and a control emulsion of agar media injected in the right arm. A negative result shows as an erythema without pain or papule formation. Positive reactions show as papules vesicles or even pustules giving rise to discomfort for several days. While the control side usually becomes normal in forty-eight hours yet in latent and tertiary syphilis the control may show almost as marked a reaction. The term "Umstimmung" is applied to this susceptibility to trauma of the skin of those having tertiary syphilis. Some cases of parasyphilitic infections which are negative to the Wassermann test give a positive luetin reaction.

Noguchi has recently demonstrated T. pallidum in all layers of the cerebral cortex except the outermost one in 12 cases out of 70 cases of general paresis examined.

In diagnosis either use the dark ground illuminator or make a thin smear from the sanious oozing after vigorous friction of the chancre with gauze, taking up this blood-stained serum on the end of a slide and smearing the surface of a second slide with the adhering material. It is in most cases more satisfactory to curet the lesion, in this way obtaining material from the areas of the thickened arteries.

In the diagnosis of cerebrospinal syphilis we use, in addition to the Wassermann test of the blood, (1) the Nonne-Apelt reaction in which about 1 c.c. of a saturated aqueous solution of ammon. sulphate is added to an equal amount of cerebrospinal fluid. If turbidity or rather opalescence appear immediately, or within three minutes, the test is positive. (2) The counting of the lymphocytes in the cerebrospinal fluid. A lymphocytosis occurs in cerebrospinal syphilis, tabes and general paresis. (3) The Wassermann test, using the cerebrospinal fluid instead of blood-serum.

T. pertenue.—An organism of similar morphology was first reported by Castellani as present in yaws. It is found in smears and sections as with T. pallidum.

A point of distinction between these spirochætes is that the T. pallidum is found in abundance in sections from a chancre about the thickened arteries in the corium, while in sections from a yaws nodule the T. pertenue is found chiefly in the region of the interpapillary pegs of the Malpighian layer of the epidermis where they bound the papillary layer of the corium.

T. pertenue has been cultivated in the same way as T. pallidum and Nichols has infected rabbits by intratesticular injection. A disease of Guam known as gangosa is possibly connected with a tertiary form of yaws. In persons who have had yaws a positive Wassermann reaction seems to be given in a higher percentage than is true for syphilis. Salvarsan is also more specific for yaws than for syphilis.

Trypanosoma.

The genus Trypanosoma has a more or less spindle-shaped body, along one border of which runs an undulating membrane. There is one flagellum bordering the membrane and projecting like a whip posteriorly.

There is a nucleus (macronucleus) and a blepharoplast (micronucleus—centrosome), the latter being located anteriorly as a chromatin staining dot or rod. From this blepharoplast the flagellum proceeds posteriorly bordering the undulating membrane and projecting freely beyond the posterior end. The nucleus is larger, nearer the posterior end, and does not stain so intensely as the blepharoplast.

Some consider that the trypanosome developed from types with a single anterior flagellum proceeding from a blepharoplast. The moving of the blepharoplast with the flagellum to the other end would make the flagellar end the anterior end. This controversy as to which is the anterior end is the cause of confusion.

T. gambiense.—This is the trypanosome causing human trypanosomiasis, the latter stage of which is known as sleeping sickness. It is from 17 to 28μ long, and from 1.5 to 2μ wide. Blepharoplast oval.

It was first discovered in smears from blood by Ford in 1901, and recognized as a trypanosome by Dutton in 1902, and observed in 1903 by Castellani in the cerebrospinal fluid of patients with sleeping sickness. It is now proposed to consider cases where trypanosomes are not present in the cerebrospinal fluid as in the first stage; when present, as in the second stage.

It is very difficult to distinguish the human trypanosome from some of the other pathogenic ones by staining methods. The immunity test is the most reliable. An animal recovered from an infection by a certain trypanosome does not possess immunity for other pathogenic ones. Novy and McNeal cultivated T. lewisi in water of condensation on blood agar at room temperature and Thomson and Sinton have recently cultivated both T. gambiense and T. rhodesiense by using rat's blood instead of rabbit's blood in the N.N.N. medium. It is present in the blood, usually in exceedingly small numbers, and in the lymphatic glands of patients. It is by puncture of the glands that we have the best means of finding the parasites. It is also found in the cerebrospinal fluid in sleeping sickness. The parasite stains readily with Wright's stain. The transmitting agent is the Glossina palpalis.

The life history of T. gambiense is not so well understood as that of certain other organisms. There seem to be certain periods when even with trypanosomes in the peripheral circulation tsetse flies do not become infected. From about 2 to 5% of flies seem to become infective in experiments. When blood containing the so-called short form of trypanosomes is ingested by G. palpalis they reach the gut and remain there unattached. From the fifth to the seventh day they seem to become scarce in the digestive tract but later they reappear in quantity. About the eighth to the eighteenth day long slender forms pass into the proventriculus and later reach the salivary glands as long slender forms. They multiply in the glands and develop into short crithidial forms which later become similar to those found in the peripheral circulation. Robertson considers that the important development takes place in the salivary glands and not in the intestine while Kleine thinks the mature forms the first to appear in the gut. It requires eighteen to twenty days or longer for the complete development and flies so infective remain so for the remainder of life.

Some authors consider types representing male, female and indifferent forms to be noted during the developmental cycle.

Other authorities think it possible that trypanosomes may encyst in the digestive tract, and so the flies transmit the disease along with their fæces. This does not seem to be possible in connection with human infections. Koch found several cases where infection had taken place by coitus. This is the method of infection in T. equiperdum, a trypanosome disease of horses.

The various trypanocidal remedies, atoxyl, arsacetine, etc., have not proven very satisfactory. One of Ehrlich's latest products, arsenophenyl-glycine, however, has given encouraging results; horses affected with surra having been cured by its use. In man it has been given in doses of 1 gram without ill effects.

T. rhodesiense.—This is a trypanosome reported for man by Stephens and Fantham. The nucleus, instead of being in the center as in T. gambiense, is quite near the blepharoplast. It is much more virulent for laboratory animals than T. gambiense. It is transmitted by G. morsitans and the developmental cycle is similar to that of T. gambiense except that it seems that the important developmental cycle occurs in the gut of the fly.

Schizotrypanum cruzi (**Trypanosoma cruzi**) Chagas, 1909.—A human trypanosomiasis found in the state of Minas Geraes, in Brazil, is caused by this protozoon. Cruz states that the specific protozoon is transmitted by a bug of the genus Conorhinus (Reduviidæ).

This trypanosome is remarkable for the large size of its blepharoplast. In length it is only a little longer than the diameter of a red cell. It is cultivable on blood agar and can be transmitted to various laboratory animals, as guinea-pig, white mice, and monkeys.

Cruz thinks that a non-sexual cycle occurs in general tissues of man but that a special sexual cycle occurs in the lung capillaries. In the lungs the parasite loses its flagellum and becomes oval in shape. Subsequently eight daughter spores develop. These spores or merozoites are liberated into the general circulation and each one penetrates a red cell and develops into an adult trypanosome. When ingested by Conorhinus they lose the flagellum and assume an oval Leishmania form, which multiply by fission. Eventually there are produced trypanosome types which get into the salivary glands and thence into man. Chiefly a disease of children with swelling of neck, axillary and groin glands, anæmia, enlarged spleen, œdema of eyelids and irregular fever. Usually fatal in children but less so in adult. In adults apt to have goiter.

Of the more important trypanosome diseases of animals may be mentioned:

1. Nagana. Pathogenic for domesticated animals in South Africa. T. brucei.
2. Surra. Pathogenic for horses in India and Philippines. T. evansi.
3. Dourine. Transmitted by coitus in horses. T. equiperdum.
4. Mal de caderas. Affects horses in South America. T. equinum.

A harmless infection, especially in sewer rats, is due to T. lewisi. Transmission of

this rat trypanosomiasis can apparently be brought about through the agency of both fleas and lice. In the flea there is apparently a developmental cycle of a duration of one week.

There are many trypanosomes in birds, fish, frogs, etc.

Trypanoplasma.

The genus Trypanoplasma has a rather large blepharoplast, from which arise two flagella. One extends forward as a free anterior flagellum, while the other projects posteriorly, running along the border of the undulating membrane. This genus is not known for man.

Leishmania.

The genus Leishmania includes three species: L. donovani, the parasite of kala azar, L. tropica, the parasite of oriental sore and L. infantum the cause of a leishmaniasis among children in northern Africa.

The disease known as ponos, which exists in the Grecian islands Spezzia and Hydra has been found by Gallé to be a leishmaniasis. Nicolle has found a disease of very young children (as a rule in the second year of life) in Tunis due to L. infantum. This protozoon morphologically resembles L. tropica but is smaller. It is found chiefly in the spleen, liver and bone marrow. The symptoms are extreme anæmia, splenic, and to a less degree, hepatic enlargement. Irregular temperature. rapid pulse and a mononuclear leukocytosis and transient œdema are also noted. It can be inoculated into the dog and monkey; other animals are practically immune.

A similar disease has been noted in Italy, Malta, and Portugal. L. infantum grows rapidly in Novy MacNeal medium, in which medium L. donovani will not grow. Furthermore inoculation of L. donovani into dogs and monkeys has been unsuccessful. These are undoubtedly different species, inasmuch as in sections of India, where tropical ulcer was common, there was no kala azar, and in Assam where kala azar prevailed there were no Leishman-Donovan bodies to be found in smears from the tropical ulcerations there present, except rarely in cases of general infection. L. tropica has been cultivated by Nicolle.

It is interesting that the parasite of kala azar cannot be cultivated except in sterile media while that of oriental sore will grow in media contaminated with cocci.

These parasites are typically intracellular, being within either polymorphonuclears, which contain only one or two of the bodies, or in large mononuclears, in which there may be as many as six. They may be packed, however, in phagocytic endothelial cells.

In kala azar smears taken during life we may find the bodies imbedded in a faintly blue staining matrix; after death and in sections of tissue such an appearance is not seen. In the spleen they are not found in the Malpighian bodies, but in the phagocytic cells lining the lymph spaces. The parasites occur in the peripheral circulation in about 80% of the cases. They abound in the liver and spleen. The parasite is oval and about $2 \times 3 \mu$. There are two distinct chromatin staining masses. The larger nucleus is more or less spherical, peripherally situated, and stains faintly,

while the smaller chromatin mass is generally rod-shaped and stains intensely. It has been recently recommended that instead of liver or splenic puncture for the demonstration of these bodies, a blister be raised and a smear from that containing many polymorphonuclears might show these bodies. The affection is characterized by a leukopænia so that it is very difficult to demonstrate the parasites in ordinary blood smears.

By cultivating the parasites obtained from splenic puncture in acidified sodium citrate solution at room temperature, Rogers succeeded in obtaining flagellated forms similar to Herpetomonas. An anterior flagellum proceeds directly from the blepharoplast. The bedbug is supposed to be the intermediary host.

Patton has recently noted that when bedbugs feed on kala-azar patients who have the L. D. bodies in their peripheral circulation that the parasites develop into the flagellate stage in the bedbug and are present in great numbers from the fifth to the eighth day. These flagellate forms change into postflagellate ones by the twelfth day and are then found in the stomach. If, however, he allowed the bedbugs to have a second feeding of human blood after the infecting feeding the flagellates disappeared within twelve hours. This is apparently an important point in epidemiology. Patton succeeded in infecting a white rat with intraperitoneal injection of splenic emulsion from a kala-azar patient.

L. infantum is transmitted from dog to dog by the dog flea, P. serraticeps and the same agent probably transfers the parasite from dog to man. Experiments would indicate that the Indian form of kala azar is not a disease which can be transmitted to dogs.

The genera Herpetomonas and Crithidia are frequently found in the alimentary tract of insects and have caused confusion in the search for developmental forms of various pathogenic flagellates in transmitting insects. In Herpetomonas, of which the type species is H. muscæ domesticæ, the body is spindle-shaped with a rather blunt flagellar end and an attenuated anterior end. In Crithidia both extremities are pointed and the blepharoplast is situated toward the center quite near the trophonucleus. In Herpetomonas the blepharoplast is near the rather blunt flagellar extremity at some distance from the nucleus.

There is no undulating membrane in either of these genera, this differentiating them from Trypanosoma.

Darling has reported from Panama a protozoon somewhat like Leishmania in which the cells of lungs, liver, spleen, and lymphatic glands contained numerous parasites about 3 to 4μ in diameter, slightly oval in outline, and containing a large and small chromatin staining mass. He has given it the name Histoplasma capsulata.

Trichomonas.

Trichomonas vaginalis.—This parasite has a fusiform body and is about 18 × 10μ. It has three flagella arising from the anterior end and an undulating membrane. It lives in vaginal mucus which has an acid reaction A change of reaction, as at menstruation, causes them to disappear. Forms similar to the T. vaginalis have been found in the intestine and in sputum from putrid bronchitis.

These flagellates are generally considered harmless, although doubt as to this is expressed by some authors.

Lamblia.

Lamblia intestinalis.—These parasites are about 10×15μ and have a pear-shaped body with a depression at the blunt anterior end. This depression enables the flagellate to attach itself to the summit of an epithelial cell. Around the depression are three pairs of flagella which are constantly in motion. Another pair of flagella project from either side of the blunt little tail-like projection. When stained, the parasites have a pyriform shape with two chromatin staining areas on either side of the anterior end. When encysted, they assume an oval shape. This parasite is generally considered as of little importance, but inasmuch as, when in great numbers in the cæcum and appendix, they may give rise to symptoms resembling appendicitis and as they are responsible for a chronic and intractable diarrhœa associated with mental and physical depression, this is undoubtedly an affection only minor in importance to amœbic infection. It is a common infection in the tropics.

Infusoria (Ciliata).

The Infusoria are the most highly developed of the Protozoa.

The bodies of Infusoria are oval and may be free or attached to a stalk-like contractile pedicle, as with Vorticella, or they may be sessile. The cilia, which are characteristic, may be markedly developed around the cytostome (mouth) and serve the purpose of directing food into the interior, while others act as locomotor organs. The body is enveloped by a cuticle which may only have one opening or slit, to serve as mouth; or it may have a second one, a cytopyge or anus. Usually the fæcal matter is ejected through a pore which may be visible only when in use. They usually have a large nucleus and a small one. Infusoria tend to encyst when conditions are unfavorable (as when water dries up in a pond). When the cilia are evenly distributed over the entire body of the ciliates we have the order Holotricha; when ciliated all over, but with more prominent cilia surrounding the peristome, we call the order Heterotricha. It is to this order that the Infusoria of man belong.

Balantidium coli.—This is the only ciliate of importance in man. It is a common parasite of hogs. It is from 60 to 100μ long by 50 to 70μ broad, and has a peristome at its anterior end which becomes narrow as it passes backward. It has an anus. The ectosarc and the endosarc are distinctly marked. The cuticle is longitudinally striated.

These parasites cause an affection similar to dysentery and may bring about a fatal termination. It is almost impossible to escape noticing the actively moving bodies if a fæcal examination is made. When encysted they are round.

Another ciliate, the B. minimum, 25 x 15μ, has also been reported for man.

Nyctotherus faba has a kidney-shaped body and is about 25 by 15μ. It has a large contractile vacuole at the posterior end. It has a large nucleus in the center with a small fusiform micronucleus lying close to it. It has only been reported once for man.

DESCRIPTION OF PLATE I.

(Kolle and Wassermann.)

Malarial Parasites.

1. Two tertian parasites about thirty-six hours old, attacked blood-corpuscles swollen.

2. Tertian parasite about thirty-six hours old; stained by Romanowsky's method. The black granule in the parasite is not pigment but chromatin. Next to it and to the left is a large lymphocyte, and under it the black spot is a blood plate.

3. Tertian parasite, division form nearby is a polynuclear leukocyte.

4. Quartan parasite, ribbon form.

5. Quartan parasite, undergoing division.

6 Tropical fever parasite. (Æstivo-autumnal.) In one blood-corpuscle may be seen a smaller, medium, and large tropical fever-ring parasite.

7. Tropical fever parasite. Gametes half-moon spherical form. Smear from bone marrow.

8. Tropical fever parasite which is preparing for division heaped up in the blood capillaries of the brain.

Asexual Forms.

9. Smaller tertian ring about twelve hours old.

10. Tertian parasite about thirty-six hours old, so-called amœboid form.

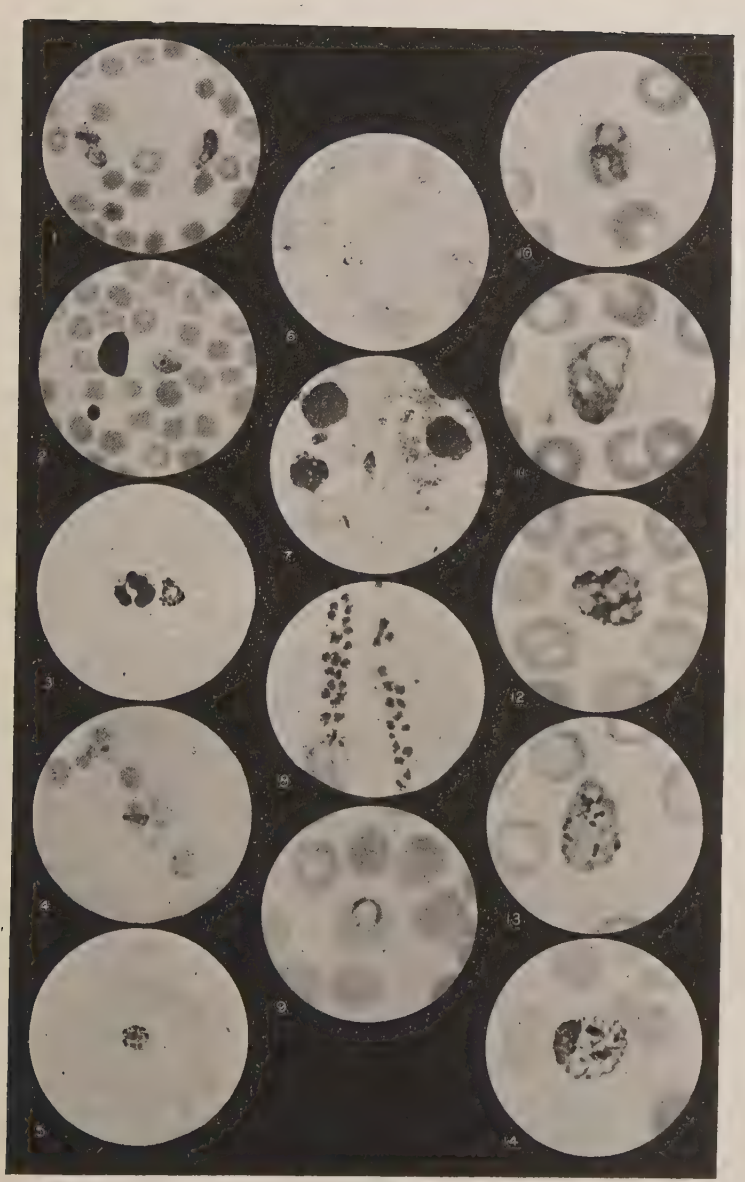
11. Tertian parasite still showing ring form forty-two hours old.

12. Tertian parasite, two hours before febrile attack. The pigment is beginning to arrange itself in streaks or lines.

13. Tertian parasite further advanced in division. Pigment collected in large quantities.

14. Further advanced in the division. (Tertian parasite.)

PLATE I.



DESCRIPTION OF PLATE II.

(Kolle and Wassermann.)

Malarial Parasites.

15. Complete division of the parasite. Typical mulberry form.

16. To the left is the completed division form, an almost developed gamete, which is to be recognized by its dispersed pigment.

17. A tertian ring parasite, small size broken up.

18. Three-fold infection with tertian parasite. The oval black granules are the chromatin granules.

19. To the left, tertian parasite with large, sharply demarked, and deeply colored chromatin granules. To the right, tertian parasite. Both thirty-six hours old. Both probably gametes.

20. Tertian parasite thirty-six hours old, ring form.

21. Tertian parasite with beginning chromatin division, with eight chromatin segments.

22. Tertian parasite chromatin division farther advanced with twelve chromatin granules, in part triangular in form.

23. Completed division figure of a tertian parasite. Twenty-two chromatin granules.

24. The young tertian parasites separating themselves from each other. The pigment remains behind in the middle.

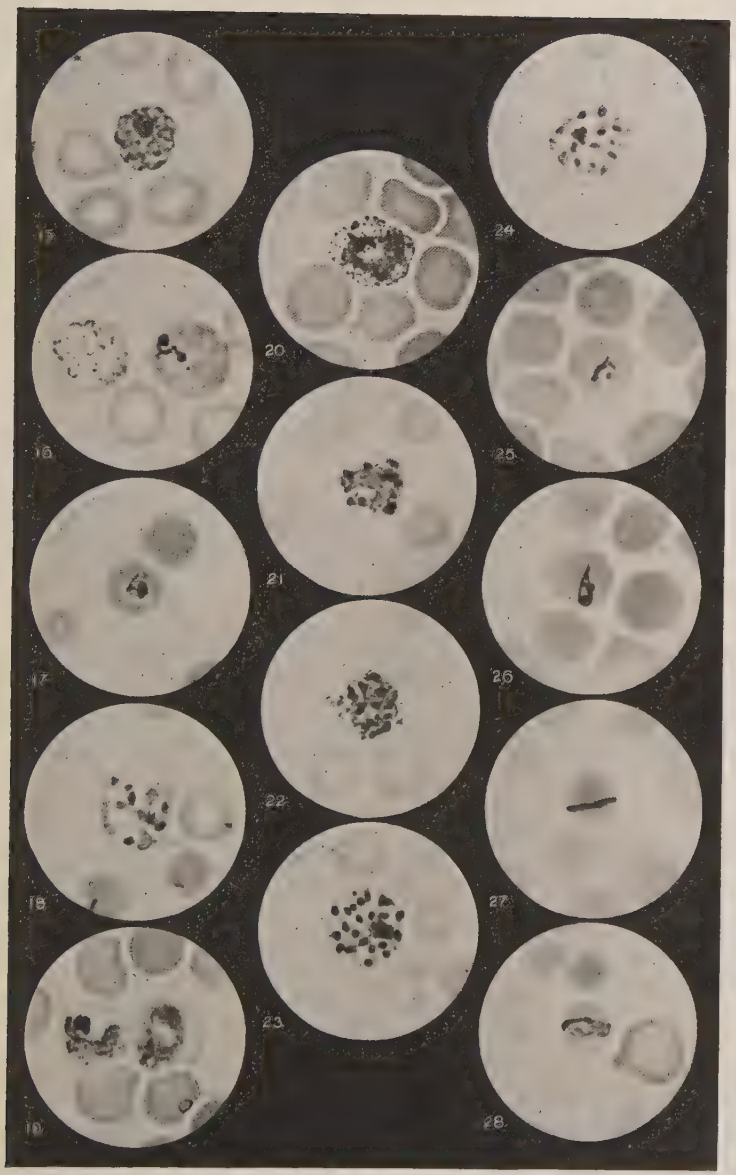
25. Quartan ring parasite, which is hard to differentiate from large tropical ring or small tertian ring.

26. Quartan ring lengthening itself.

27. Small quartan ribbon form.

28. The quartan ribbon increases in width. The dark places consist almost entirely of pigment.

PLATE II.



or C. oviforme. It is most frequently found in the epithelium of the bile ducts. It has very rarely been reported for man. In these cases (about five) cysts of the liver have been found containing coccidia. The parasite is about 40 × 20μ, and is oval in shape with a double outlined integument. The sporozoites, which form inside, are falciform in shape. These escape and enter fresh epithelial cells, and thus the process of schizogony goes on. The parasites of the liver are larger than those found in the intestines, these latter being only about 30×15μ. In the fæces the form most often found is the oocyst, about 40 × 20μ. Infection takes place by ingestion of the oocyst.

Isospora bigemina.—This parasite, formerly called the Coccidium bigeminum, lives in the intestinal villi of dogs and cats. It is about 12 × 8μ. and shows a highly refractile envelope (oocyst) containing two biscuit-shaped sporocysts within each of which are four sporozoites. It has been reported for man three times.

Hæmosporidia.

Of the Sporozoa found in the blood (Hæmosporidia), the **malarial parasites** are the only ones connected with disease in man.

In addition to man, infections with parasites of a similar nature are found in monkeys (Plasmodium kochi; the sexual forms alone seem to be present), in birds (Hæmamœba relicta; this organism is usually designated Proteosoma). An infection of crows and pigeons of like nature is Halteridium. Numerous hæmosporidia have been reported for bats, various other mammals, tortoises, lizards, etc.

The life history of the malarial parasite is one of the most interesting chapters in medicine. Laveran discovered the parasite in 1880. In 1885, Golgi noted that sporulation occurred simultaneously at time of malarial paroxysm. Koch, Golgi, and Celli demonstrated existence of different species for different types of fever. King and Laveran (1884) considered possibility of mosquito transmission. Manson (1894) formulated hypothesis that gametes were destined to undergo development in the mosquito from observing that flagellated bodies only appeared some time after the blood was withdrawn.

Ross (1895) demonstrated that flagellation takes place in the stomach of the mosquito. McCallum (1897) saw fertilization of macrogametes by microgametes of Halteridium. Opie recognized differences in sexual characteristics.

Ross (1898) demonstrated life cycle of bird malaria (Proteosoma), showing formation of zygotes and presence of sporozoites in salivary glands. Grassi and Bignami proved the cycle for Anophelinæ for human malaria. In 1900 (Sambon and Low), infected mosquitoes from Italy were sent to London, where, by biting, they infected two persons.

Life History.—When man is at first infected by sporozoites we have starting up a nonsexual cycle which is completed in from forty-eight to seventy-two hours, according to the species of parasite. The falciform sporozoite bores into a red cell, assumes a round shape and continues to enlarge (schizont). Approaching maturity, it shows division into a varying number of spore-like bodies. At this stage the parasite is

DESCRIPTION OF PLATE III.

(Kolle and Wassermann.)

Malarial Parasite.

29, 30, 31. The quartan ribbon increases in width. The dark places consist almost entirely of pigment.

32. Beginning division of the quartan parasite and the black spot in the middle is the collected pigment.

33. Quartan ring.

34. Double infection with quartan parasites.

35. Wide quartan band. The fine black stippling in the upper half of the parasite is pigment.

36. Beginning division of the quartan parasite. The chromatin (black fleck) is split into four parts.

37. Division advanced, quartan parasites.

38. Typical division figure of the quartan parasite.

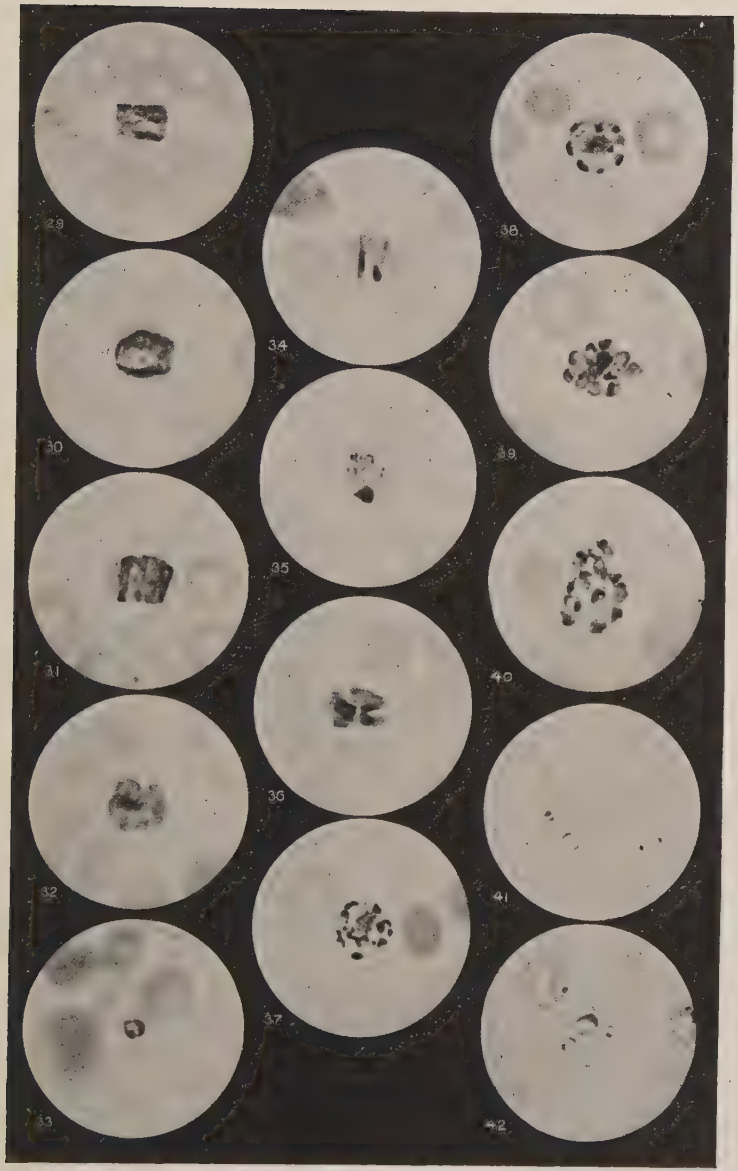
39. Finished division of the quartan parasite. Ten young parasites, pigment in the middle.

40. Young parasites separated from one another.

41. Small and medium tropical ring, the latter in a transition stage to a large tropical ring.

42. Small, medium and large tropical ring, together in one corpuscle.

PLATE III.



termed a merocyte. When the merocyte ruptures, these spore-like bodies or merozoites enter a fresh cell and develop as before.

At the time that the merocyte ruptures it is supposed that a toxin is given off which causes the malarial paroxysm. The cycle goes on by geometric progression from the first introduction of the sporozoite, but it is usually about two weeks before a sufficient number of merocytes rupture simultaneously to produce sufficient toxin for symptoms (period of incubation). This cycle is termed schizogony.

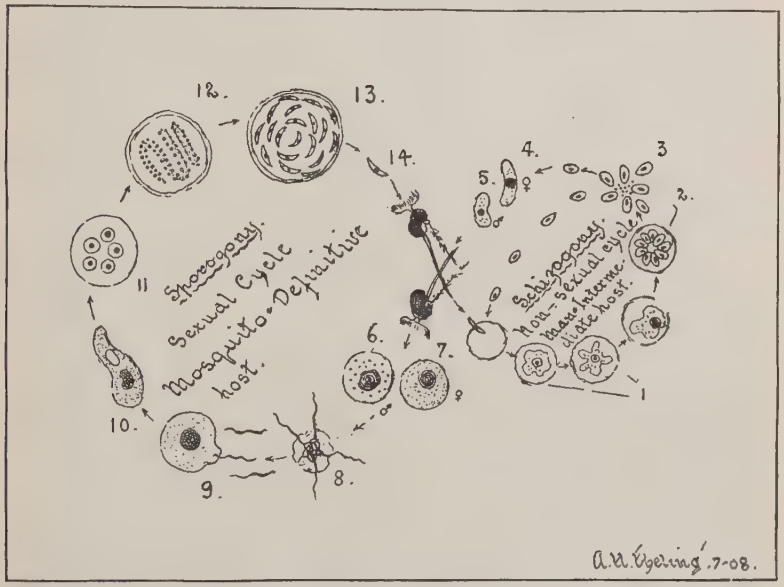


FIG. 62.—Sexual and nonsexual cycle of malaria. 1, Schizonts; 2, merocyte; 3, merozoites; 4, macrogamete; 5, microgametocyte; 6, and 7, gametes in stomach of mosquito; 8, microgametocyte throwing off microgametes; 9, microgamete fertilizing macrogamete; 10, vermiculus or zygote; 11 and 12, zygotes; 13, zygote distended with sporozoites; 14, sporozoites.

After a varying time, whether by reason of necessity for renewal of vigor of the parasite by a respite from sporulation, or whether from a standpoint of survival of the species, sexual forms (gametes) develop. Some think that sporozoites of sexual and nonsexual characteristics are injected at the same time. It is usually considered, however, that sexual forms develop from pre-existing nonsexual parasites.

These gametes show two types: the one which contains more pigment, has less chromatin, and stains more deeply blue is the female—a macrogamete; the other with more chromatin, less pigment, and staining

DESCRIPTION OF PLATE IV.

(Kolle and Wassermann.)

Malarial Parasite.

43. To the left a young (spore) tropical parasite. To the right a medium and large tropical parasite.

44. An almost fully developed tropical parasite. The black granules are pigment heaps.

45. Young parasites separated from one another. Broken up division forms twenty-one new parasites.

46. To the left a red blood-corpuscle with basophilic, karyochromatophilic granules. Prototype of malarial parasite. On the right a red blood-corpuscle with remains of nucleus.

Sexual Forms or Gametes.

47. An earlier quartan gamete (macrogamete in sphere form), female.

48. An earlier quartan gamete (microgametocyte), male.

49. Tertian gamete, male form (microgametocyte).

50. Tertian gamete, female (macrogamete).

51. Tertian gamete (microgametocyte) still within a red blood-corpuscle.

52. Macrogamete tertian within a red blood-corpuscle.

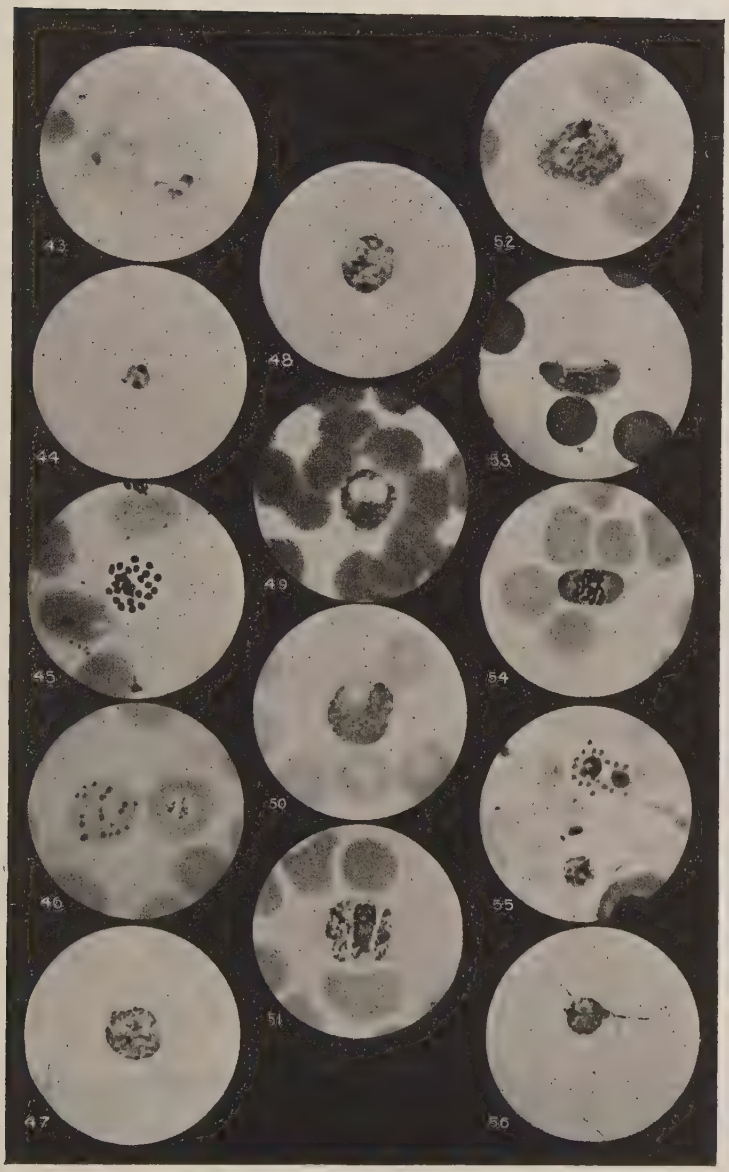
53. Tropical fever. (Æstivo-autumnal) gamete, half-moon (crescent) still lying in a red blood-corpuscle. In the middle is the pigment. The concave side of the crescent is spanned by the border of the red blood-corpuscle.

54. Gamete, tropical fever parasite.

55. Gamete of tropical fever parasite heavily pigmented.

56. Gamete of the tropical fever parasite (flagellated form), microgametocyte sending out microgametes (flagella or spermatozoa).

PLATE IV.



grayish-green rather than blue is the male—a microgametocyte. When the gametes are taken into the stomach of the Anophelinæ, the male cell throws off spermatozoa-like projections, which have an active lashing movement and break off from the now useless cell carrier and are thereafter termed microgametes. These fertilize the macrogametes and this body now becomes a zygote.

By a boring-like movement the zygote goes through the walls of the mosquito's stomach, stopping just under the outer epithelial layer of the stomach or mid-gut. It continues to enlarge until about the end of one week it has grown to be about 60μ in diameter and has become packed with hundreds of delicate falciform bodies.

Zygotes of benign tertian show little rod-like particles of yellowish pigment—those of malignant tertian black clumps, which, however, are not so coarse as those of quartan.

The mature zygote now ruptures and the sporozoites are thrown off into the body cavity. They make their way to the salivary glands and thence, by way of the veneno-salivary duct in the hypopharynx, they are introduced into the circulation of the person bitten by the mosquito, and start a nonsexual cycle. As the sexual life takes place in the mosquito, this insect is the definitive host—man is only the intermediary host.

It must be remembered that only certain genera and species of Anophelinæ are known malaria transmitters; thus Stephens and Christophers, in dissecting 496 mosquitoes of the species M. rossi, did not find a single gland infected with sporozoites. With M. culicifacies, however, twelve in 259 showed infection.

This is one of the methods of determining the endemicity of malaria or the malarial index. There are two other methods: 1. by noting the prevalence of enlarged spleen, and 2. by determining the number of inhabitants showing malarial parasites in the blood. This index is best determined from children between two and ten years of age, as children under two years show too high a proportion of parasites in the peripheral blood while those over ten years of age show too great an incidence of enlarged spleens.

There are three species of malarial parasites: 1. the Plasmodium vivax, that of benign tertian—cycle, forty-eight hours; 2. the Plasmodium malariæ, that of quartan—cycle, seventy-two hours; and 3. the Plasmodium falciparum, that of æstivo-autumnal or malignant tertian—cycle of forty-eight hours.

Variations in cycles may be produced by infected mosquitoes biting on successive nights, so that one crop will mature and sporulate twenty-four hours before the second. This would give a quotidian type of fever. In æstivo-autumnal infections anticipation and retardation in the sporulation cause a very protracted paroxysm, lasting eighteen to thirty-six hours; this tends to give a continued or remittent fever instead of the characteristic type.

Unstained Specimen (Fresh Blood).

	P. vivax. (Benign tertian.)	P. malariæ. (Quartan.)	P. falciparum. (Malignant tertian) (Æstivo-autumnal.)
Character of the infected red cell.	Swollen and light in color after eighteen hours.	About the size and color of a normal red cell.	Tendency to distortion of red cell rather than crenation. Shriveled appearance. (Brassy color.)
Character of young schizont.	Amœboid outline. Hyaline. Rarely more than one in r.c. Active amœboid movement. One third diam. of r. c.	Frosted glass disc. Very slight amœboid motion.	Small, distinctly round, crater-like dots not more than one-sixth diameter of red cell. Two to four parasites in one red cell common.
Character of mature schizont.	Amœboid outline. No amœboid movement.	Rather oval in shape. Sluggish movement of peripherally placed coarse black pigment.	Only seen in overwhelming infection. Have scanty fine black pigment clumped together.
Pigment.	Fine yellow brown granules which show active motion in one-half grown schizont. Motion ceases in full-grown schizont.	Coarse almost black. Shows movement only in young to half-grown schizont.	Pigmented schizonts very rare in periph. circulation except in overwhelming infections. Tend to clump as excentric pigment mass blocks.

Stained Specimen.

	P. vivax. (Benign tertian.)	P. malariæ. (Quartan.)	P. falciparum. (Malignant tertian) (Æstivo-autumnal.)
Character of infected red cell.	Larger and lighter pink than normal red cell. Shows "Schüffner's dots."	About normal size and staining.	Shows distortion and some polychromatophilia and stippling. Rarely we have coarse cleft-like reddish dots —Maurer's spots.

STAINED SPECIMEN.—(*Continued.*)

	P. vivax. (Benign tertian)	P. malariæ (Quartan.)	P. falciparum (Malignant tertian) (Æstivo-autumnal.)
Character of young schizont.	Chromatin mass usually single and situated in line with the ring of the irregularly outlined blue parasite.	Rather thick round rings which soon tend to show as equatorial bands.	Very small sharp hair-like rings, with a chromatin mass protruding from the ring. Often appears on periphery of red cell as a curved blue line with prominent chromatin dot. Frequently two chromatin dots.
Character of half-grown schizont.	Vacuolated looped-like body with single chromatin aggregation. Schüffners dots.	More marked band forms stretching across r.b.c.	Not often found in peripheral circulation. Chromatin still compact.
Character of mature schizont.	Fine pigment rather evenly distributed in irregularly outlined parasite.	Coarse pigment rather peripherally arranged in an oval parasite.	Very rarely seen in peripheral circulation in ordinary infection. Pigment clumps early.
Character of merocyte.	Irregular division into fifteen or more spore-like chromatin dot segments.	Rather regular division into eight or ten merozoites—Daisy.	Sporulation occurs in spleen, brain, etc. Rarely in peripheral circulation. Eight to ten chromatin staining merozoites.
Character of macrogamete.	Round deep blue. Abundant, rather coarse pigment, chromatin at periphery.	Round, similar to P. vivax but smaller.	Crescentic, deep blue, pigment clumped at center, chromatin scanty and in center.
Character of microgametocyte.	Round, light green-blue, pigment less abundant, chromatin abundant and located centrally or in a band.	Round like P. vivax.	More sausage-shaped than crescent. Light blue. Pigment scattered throughout. Chromatin scattered.

In full grown schizonts we find the chromatin in separate aggregations throughout the parasite while the pigment is clumped. In gametes the pigment is scattered and the chromatin is in a single mass.

If many young ring forms are present during pyrexia it is probable that the infection is E.A.

In parthenogenesis, as observed in P. vivax, the nonsexual forms and the males die off leaving only the female forms. The nucleus divides into a dense and light portion. The latter degenerates and the former goes on to merozoite formation.

This is Schaudinn's explanation of relapses. Another explanation of latent malaria is by conjugation of two ring forms.

In the diagnosis of malaria one should always examine both a fresh specimen and a stained one, as each method gives valuable information in differentiating species. When time will not permit the examination by both methods, always use the smear stained by Wright's stain, as the small peripherally situated rings of æstivo-autumnal fever may escape notice in a fresh specimen.

For the cultivation of malarial parasites (**Bass**) the blood in 10 to 20 c.c. quantities is taken from the patient's vein and received in a centrifuge tube which contains 1/10 c.c. of 50% glucose solution. A glass rod, or piece of tubing, extending to the bottom of the centrifuge tube is used to defibrinate the blood. After centrifugalizing there should be at least 1 inch of serum above the cell sediment. The parasites develop in the upper cell layer about 1/50 to 1/20 inch from the top. All of the parasites contained in deeper lying red cells die. To observe the development, red cells from this upper 1/20-inch portion are drawn up with a capillary bulb pipette.

Should the cultivation of more than one generation be desired, the leukocyte upper layer must be carefully pipetted off, as the leukocytes immediately destroy the merozoites. Only the parasites within red cells escape phagocytosis. Sexual parasites are much more resistant, and the authors think they observed parthenogenesis. The temperature should be from 40 to 41° C. and strict anaerobic conditions observed. Æstivo-autumnal organisms are more resistant than benign tertian ones. Dextrose seems to be an essential for the development of the parasites.

Bass considers that P. vivax has a flat amœboid like structure which enables it to squeeze through the brain capillaries while adult schizonts of P. falciparum have a solid oval form which causes them to be caught in the capillaries.

Belonging like the malarial parasite to the Hæmosporidia we have a group of parasites known as the PIROPLASMS. The correct name for these parasites is *Babesia* but they are better known under the name *Piroplasma*. They are minute organisms, usually pear or rod shape, which invade the red corpuscles. They produce no pigment but destroy the corpuscle and set free the Hb. which is excreted in enormous amounts by the kidneys. It is this which gives the name redwater to the better known Texas fever of cattle. Organisms of this kind have been thought of in connection with blackwater fever of man. Seidelin has claimed that a parasite of similar nature, *Paraplasma flavigenum*, was the cause of yellow fever.

At one time spotted fever of the Rocky Mountains was supposed to be due to a parasite named *Babesia hominis*.

Sarcosporidia.

Sarcosporidia are sporozoa found in the striped muscles of various mammals and birds. They are common in the pig and mouse and have been reported for man in three well-authenticated cases. In the last, Darling found these protozoa in the biceps muscle of a negro patient in Panama. In Baraban's case the laryngeal muscles at autopsy were found to show cysts about 1/15 inch long which contained sickle-shape sporozoites about 9μ long.

They are known also as Miescher's tubes when in muscle fibers. They are divided into three genera: Miescheria and Sarcocystis when parasitic in muscle fiber; Balbiania, when parasitic in the in tervening connective tissue of the muscles. The method of transmission is unknown. In some places more than 50% of the sheep and pigs may show infection.

Miescheria has a thin membrane surrounding the cyst while that of Sarcocystis is thickened and radially striated by small canaliculi.

As the young trophozoite grows nuclei increase and a definite membrane forms which the sporoblasts eventually fill. According to Minchin the Sarcosporidia contain only one genus, Sarcocystis. It is never parasitic for invertebrate hosts and while occasionally found in birds and reptiles it is pre-eminently a parasite of the higher vertebrates. As a rule, they are harmless parasites but the Sarcocystis muris is very pathogenic for the mouse. Closely related to the order Sarcosporidia is the parasite *Rhinosporidium kinealyi*.

Rhinosporidium kinealyi.—It causes pedunculated tumors of nasal cavity. The pansporoblasts enlarge in the center of the connective tissue of the nasal polyp and contain about 12 sporoblasts. When mature the cystic-like polyp bursts and the sporoblasts are liberated to extend the infection.

Fig. 63.—Miescher's sac from the musculature of a hog. ×30 diameters. (*After Ostertag.*)

Chlamydozoa.

These organisms are generally considered as being protozoal in nature and as a rule belong to the filterable viruses, which is the designation for the infectious principles of those diseases, in which filtration of defibrinated blood or serum through a Berkefeld filter capable of holding back so small an organism as the M. melitensis, does not prevent the infection being transmitted when introduced by the proper atrium of infection. The Chlamydozoa are also characterized by the occurrence of "cell inclusions."

The best known infections of this group of diseases in man are smallpox, vaccinia, rabies, trachoma, molluscum contagiosum, and foot and mouth disease. There are many such infections in other animals. The cell inclusions are regarded as products of cellular reaction to a virus which is more or less impossible of demonstration. The discovery of exceedingly minute granules in some of these diseases, as in variola and trachoma, has suggested that, as a reaction to the invasion by such a granule, the cell throws an enveloping mantle about the invading particle. To designate this we use the name Chlamydozoa.

The generic name Cytorrhyctes has been applied to certain of these viruses, thus C. vacciniæ develops within the epithelial cells of stratified epithelium. In vaccinia, Councilman and his colleagues consider that the development only takes place in the cytoplasm of the cell. In variola, however, the developmental cycle affects the nucleus.

Cytorrhyctes luis, reported as the cause of syphilis, sporulates in the blood-vessels and in the connective tissue, not in epithelial cells.

Cytorrhyctes scarlatinæ was reported by Mallory to have been found in the skin in four cases of scarlet fever.

CHAPTER XVII.

FLAT WORMS.

Classification of the Platyhelminthes (Flat Worms).

Class	Family	Genus	Species
Trematoda	Fasciolidæ	Fasciola	F. hepatica
		Fascioletta	F. ilocana
		Fasciolopsis	F. buski
		Dicrocœlium	D. lanceatum
		Paragonimus	P. westermanii
		Opisthorchis	O. felineus
		Clonorchis	C. sinensis
			C. endemicus
		Heterophyes	H. heterophyes
	Paramphistomidæ	Cladorchis	C. watsoni
		Gastrodiscus	G. hominis
	Schistosomidæ	Schistosomum	S. hæmatobium
			S. japonicum
			S. mansoni
Cestoda	Dibothriocephalidæ	Dibothriocephalus	D. latus
		Diplogonoporus	D. grandis
	Tæniidæ	Dipylidium	D. caninum
		Hymenolepis	H. nana
			H. diminuta
		Tænia	T. solium
			T. saginata
		Davainea	D. madagascariensis

Note.—Two larval Tæniidæ are found in man (Cysticercus cellulosæ and Echinococcus polymorphus).

Also two larval Dibothriocephalidæ (Sparganum mansoni and Sparganum prolifer).

Two parasites often referred to as ophthalmic flukes have been reported lying between the crystalline lens and its membrane. They have been considered as possibly trematode larvæ. Distomum ophthalmobium was found in 1850 in the eye of a child and Monostoma lentis in the eye of an old woman.

TREMATODES OR FLUKES.

Flukes are generally leaf-like in outline, rarely cylindrical, and exhibit marked variation in size and shape. They are nonsegmented and do

not have cilia on ectoderm. Very characteristic of them is the possession of suckers by which they hold on to the skin or alimentary system of their host.

They are divided into two orders: 1. the Monogenea in which the egg gives rise to a larva which later becomes the adult and 2. the Digenea. It is to this latter that the flukes parasitic in man belong. This order is characterized by the fact that the larva becomes parasitic in some second animal and then gives rise to a second generation of larvæ which latter develop into adults.

The largest human fluke, Fasciolopsis buski, is from two to three inches (50 to 75 mm.) in length, while the Heterophyes heterophyes is less than 1/12 of an inch (2 mm.) in length. The most important fluke, the liver fluke, Clonorchis endemicus, is flat and almost transparent, while the almost equally important lung fluke, the Paragonimus westermanii, is oval, almost round and reddish-brown in color. With the exception of the Schistosomidæ, all flukes are hermaphrodites, and, with the exception of this family, all flukes have operculated eggs. The only other operculated (with a lid) eggs we meet with in man are those of the Dibothriocephalidæ.

The three important families of flukes parasitic for man are: 1. Paramphistomidæ—flukes with two suckers situated at either extremity. 2. Fasciolidæ—flukes with two suckers, one terminal, the other adjacent to it and situated ventrally. This family includes the important genera Fasciola, Opisthorchis, Dicrocœlium, Fasciolopsis, and Paragonimus. In Paragonimus and Heterophyes the genital pore is posterior to the acetabulum, in the other genera it is anterior. Fasciola has a dendritic intestinal canal which is not the case with Clonorchis, Fasciolopsis, Fascioletta, Opisthorchis and Dicrocœlium. In Dicrocœlium the testicles are anterior to the uterus, in Opisthorchis, Clonorchis, Fasciolopsis and Fascioletta they are posterior. Fasciolopsis and Clonorchis have branched testicles (the former a very large fluke-Clonorchis of medium size) while those of Opisthorchis are lobed.

3. Schistosomidæ: In this family we have a leaf-like male which by a folding in of its sides makes a channel for the thread-like female. The sexes are separate, not hermaphroditic as with the Fasciolidæ and Paramaphistomidæ.

Flukes have two suckers which, except in the Paramphistomidæ, are quite near each other—one is termed the oral sucker and the other the ventral sucker or acetabulum. The intestinal tract consists of a pharynx, proceeding from the oral sucker, which bifurcates and terminates in blind intestinal cæca.

At the posterior extremity is an excretory pore which is at the termination of a duct which divides into ramifying branches. This is the water-vascular system. The testes, of various shapes and relations to the uterus, are more or less centrally situated and have vasa deferentia. In some flukes the receptaculum seminis is a

conspicuous organ. The vitellaria are bilateral branching glands which pour nutrient material into the ootype. It is in the ootype that the eggs are formed, and opening into it we have the adjacent ovary. The shell gland is near the ovary.

A canal, known as Laurer's canal, leads from the ootype to the exterior, the function of which is in question. It is probable that as trematodes have no spermatheca, the spermatozoa from other flukes enter by way of this canal. The life history of the important human flukes is unknown. It is supposed that this, in a measure, may resemble that of the common liver-fluke of sheep (sheep rot). In this the eggs containing a ciliated embryo (miracidium) pass out in the fæces. This embryo is hatched out and, gaining the water, swims about actively until it reaches some suitable mollusk (Limnæa truncatula). By means of a pointed end, it bores its way into the body of the gasteropod and in the pulmonary chamber becomes a bag-like structure (the sporocyst) from the germinal cells of which develop a creature with an alimentary canal (redia). The rediæ tend to break out of the sporocyst and wander to the liver of the snail. These rediæ may give rise to a second generation of rediæ.

From the rediæ minute little worms resembling adult flukes in possessing suckers, but differing in the possession of a tail, develop (cercaria). Having reached maturity, these cercariæ leave the rediæ, and, as in case of Fasciola hepatica, lose the tail, become encysted on blades of grass, to be eaten by sheep and again commence the cycle. The encysted cercariæ develop into adult liver flukes. It is probable that with many flukes the cercariæ enter some host, as mollusk, insect, or fish, and that it is by eating such animals as food that man becomes infected. Looss thinks it possible that the miracidium of Schistosomum hæmatobium may bore its way directly into man, as do the larvæ of the hookworm. Manson also suggests that the reporting by Musgrave of 100 mature lung flukes in a psoas abscess makes it very probable that these parasites entered the body as miracidia. The idea in China is that the infection with the common liver fluke of man is brought about by eating fish. Fluke disease is generally known as distomatosis or distomiasis.

Liver Flukes.

Fasciola hepatica (Distomum hepaticum).—This fluke, while of enormous economic importance by reason of destruction of sheep, has only been reported twenty-three times in man, and in these instances does not seem to have occasioned marked symptoms.

It has a cone-shaped anterior projection and is about 1 1/4 inch (30 mm.) long. The intestinal canal, as well as the testicles, is branched. There is, however, a possible importance of F. hepatica in connection with a peculiar affection known as "halzoun." This results from the eating of raw goat-liver, and it is supposed that the flukes crawl up from the stomach and, entering the larynx or attaching themselves about the glottis, produce the asphyxia characteristic of the disease.

Dicrocœlium lanceatum.—This has only been reported seven times in man. The symptoms are unimportant. The fluke is about 1/3 of an inch (8 mm.) long, with testicles anterior to the uterus.

Clonorchis endemicus (Opisthorchis sinensis).—This fluke and the C. sinensis are the most important of the human liver flukes. Until recently these flukes were known as Opisthorchis sinensis.

Looss has separated this genus from Opisthorchis principally by the characteristic of branching testicles—those of Opisthorchis being lobed. This fluke is very common in China and Japan—in certain sections of Japan 20% of the population being infected. This fluke is about 1/4 to 1/2 inch (8 mm.) long and C. sinensis about 3/4 of an inch long and 1/6 of an inch broad (16 × 4 mm.) When squeezed

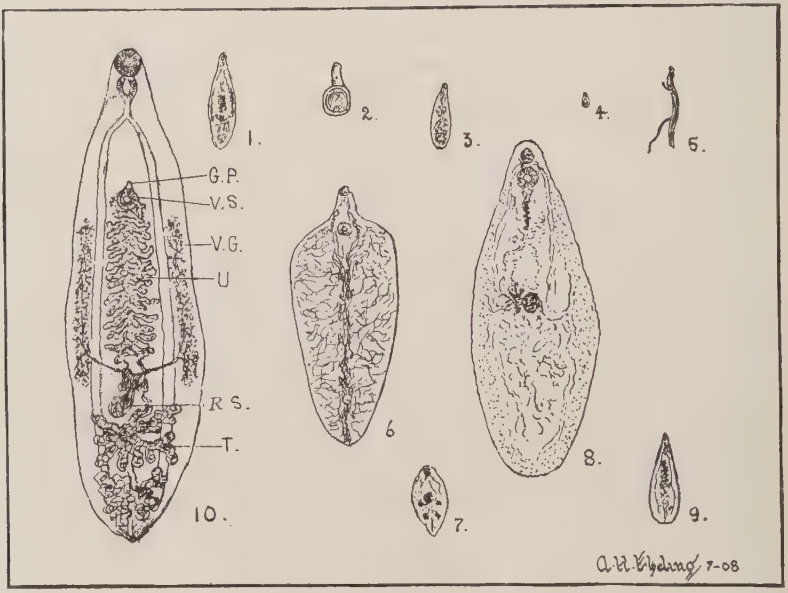


FIG. 64.—Trematodes of man, natural size. 1, Clonorchis endemicus (Opisthorchis sinensis); 2, Gastrodiscus hominis; 3, Dicrocœlium lanceatum; 4, Heterophyes heterophyes; 5, Schistosomum hæmatobium; 6, Fasciola hepatica; 7, Paragonimus westermanii; 8, Fasciolopsis buski; 9, Opisthorchis felineus; 10, anatomy of C. endemicus (enlarged). G. P., genital pore; V. S., ventral sucker; V. G., vitelline glands; R. S., receptaculum seminis; T., branched testicles.

out of the thickened bile ducts it is so transparent and glairy as almost to resemble glairy mucus. As many as 4000 of these parasites have been found in a case, chiefly in the liver, but at times in the pancreas. This fluke is supposed to produce most serious symptoms, as indigestion, swelling and tenderness of liver, ascites, œdema, and a fatal cachexia. As a matter of fact, many physicians in China attribute very little pathogenic importance to it. The disease is diagnosed by the presence of the ova in the stools. The source of infection is probably through the eating of uncooked fish.

Kobayashi has examined various mollusks and fish for trematode larvæ. He

succeeded in infecting nine kittens and two cats by feeding them with certain fresh-water fishes whose flesh contained trematode larvæ. These fish were found in districts where human distomiasis was common. The view is taken that the two species of Clonorchis are identical.

Opisthorchis felineus.—This fluke is smaller than the C. endemicus, and is a common parasite of the gall bladder and bile ducts of cats. There are two lobed testicles in this species instead of dendritic ones as in C. endemicus. In certain parts of Siberia the parasite is found in more than 6% of the human autopsies. The symptoms are similar to those caused by C. endemicus.

Other liver flukes of less importance which have been reported for man are: 1. Opisthorchis noverca. This was found in bile ducts of two natives of Calcutta. It was lancet-shaped and covered with spines.

2. Metorchis truncatus: This is a small fluke, 1/12 inch (2 mm.) long, squarely cut across at its posterior end and covered with spines. This was possibly found once in man.

Intestinal Flukes.

Cladorchis watsoni (**Amphistomum watsoni**).—This fluke is about 1/3 of an inch (8 mm.) long, of oval outline but broader at posterior end and has an indistinct oral sucker and a large sucker at the other end. This parasite has only been reported once. Eggs, 125 × 75μ.

Gastrodiscus hominis (**Amphistomum hominis**).—This fluke is about 1/4 of an inch (6 mm.) long and has a disc-like acetabulum about 1/6 of an inch in diameter from which proceeds a teat-like projection, bearing an oral sucker. While it has only been reported twice for man, indications are that it is probably fairly common in India and Assam. Eggs, 150 × 72μ.

Fasciolopsis buski (**Distomum crassum**).—This is probably a rather common parasite in India, as Dobson found the eggs in 1% of the stools of more than 1000 coolies. The fluke is from 2 to 3 inches (40 to 70 mm.) in length and about 1/2 of an inch (12 mm.) in breadth. It is thick, brown in color, and has a very large acetabulum, three times the size of the oral sucker and located almost adjacent to it. The branched ovary and shell gland lie in the center with the branched testicles posterior. The coiled uterus is anterior to the testicles. Eggs, 125 × 75μ. These parasites cause dyspeptic symptoms and an irregular diarrhœa. It is also called Distomum crassum. F. rathouisi is now considered to have been a shrunken F. buski, as it seems to be anatomically similar to F. buski. Kwan's fluke reported from Hong Kong, was possibly F. buski.

Heterophyes heterophyes (**Cotylogonimus heterophyes**).—This exceedingly small fluke (2 × 0.5 mm.), which can be recognized by its small size (less than 1/12 of an inch long) and large, prominent acetabulum, was formerly supposed to be rare. The oral sucker is much smaller than the acetabulum. The elliptical testicles lie at the extreme posterior end. Cuticle has scale-like spines. The eggs are 30 × 17μ. Very characteristic of this genus is the large sucker-like genital pore just below and to one side of the acetabulum. Looss has shown that it is quite common in Egypt, he having found it twice in Alexandria in nine autopsies. The parasites occupy the ileum. It is common in dogs.

Fascioletta ilocana.—This is a small fluke, about 1/4 inch (6 mm.) long. There are two massive testicles in the posterior part of body. The acetabulum is promi. nent. The egg of this small fluke is quite large (100μ) and has an operculum. These trematodes were found by Garrison in five natives of Luzon, P. I., after treatment with male fern.

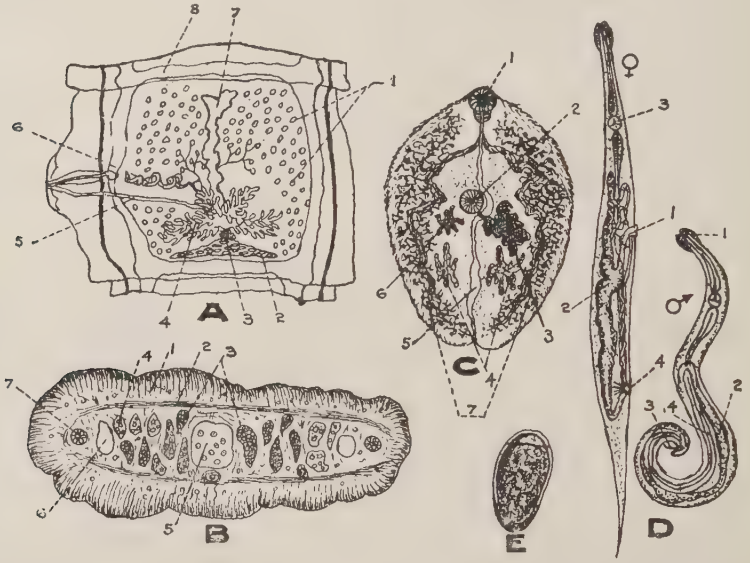


FIG. 65.—Anatomy of a tape-worm, Tænia solium (A., longitudinal, B., cross section); a fluke, Paragonimus westermanii (C)., male and female nematode, Oxyuris vermicularis (D.). A. 1, Testes; 2, yolk glands; 3, shell glands; 4, ovary; 5, vagina; 6, vas deferens; 7, uterus before branching; 8, water-vascular system. B. 1, Cuticle; 2, circular muscle; 3, ovary; 4, testes; 5, uterus; 6, excretory canal; 7, nerve cord. C. 1, Oral sucker; 2, acetabulum; 3, uterus; 4, testes; 5, excretory canal; 6, ovary; 7, yolk glands. D. (a) Female. 1, Vulva; 2, uterus; 3, bulb of œsophagus; 4, anus; (b) Male. 1, Bulbous mouth end; 2, testes; 3, spicule; 4, alimentary canal. E. Egg of P. westermanii.

Lung Flukes.

Paragonimus westermanii (Distoma ringeri).—In certain parts of Japan and Formosa it is estimated that as many as 10% of the inhabitants may harbor this parasite.

It is also common in China, and recently many cases have been reported in the Philippines. Dr. Stiles states that around Cincinnati, Ohio, there was at one time quite a heavy infection among the hogs, so that it may be that certain cases diagnosed in man as pulmonary tuberculosis are paragonimiasis.

It is popularly known as endemic hæmoptysis on account of the accompanying symptoms of chronic cough and expectoration of a rusty-brown sputum. After violent exertion, and at times without manifest reason, attacks of hæmoptysis of varying degrees of severity come on. The characteristic ova are constant in the sputum and establish the diagnosis. The fluke itself is a little more than 1/3 of an inch (8 mm.) long and is almost round on transverse section, there being, however, some flattening of the ventral surface. The acetabulum is conspicuous and opens just anterior to the middle of the ventral surface. Eggs about 90 x 65 μ.

The branched testicles are posterior to the laterally placed uterus and the genital pore opens below the acetabulum. The branched ovary is opposite the uterus on the other side.

It is rather flesh-like in appearance and is covered with scale-like spines. The flukes are usually found in tunnels in the lungs, the walls of which are of thickened connective tissue. There may be also cysts formed from the breaking down of adjacent tunnel walls. In addition to lung infection with this fluke, brain, liver, and intestinal infections may be found. Musgrave was the first one to call attention to the frequency of general infection with this parasite (paragonimiasis) in the Philippines. He found it in seventeen cases in one year. The life history, beyond the stage of miracidium, is unknown.

Another fluke which has been reported from the lung is Fasciola gigantea (very similar to F. hepatica). This was coughed up by a French officer who had been in Africa.

Blood Flukes.

Schistosomum hæmatobium.—Flukes of the circulatory system are of great importance in Egypt, South Africa, Japan, and the West Indies. The disease is named bilharziasis after Bilharz who in 1851 first associated the parasite and the disease.

It seems probable that there are at least three human species, differentiated principally by the appearance of the egg. In the blood-fluke disease of Egypt, (S. hæmatobium), the parasite chiefly infects the bladder and the egg has a terminal spine. The terminal-spined ovum is also found in the rectum and in the fæces. In the West Indies, as shown by the reports of Surgeon Holcomb from Porto Rico, rectal bilharziasis is rather common. In these cases the egg is practically always lateral-spined. Looss thinks that the lateral-spined egg is the product of an unfertilized female S. hæmatobium. These flukes differ from other human flukes in possessing nonoperculated eggs as well as in having the sexes separate. The adults of this species, the S. mansoni, are scarcely, if at all, to be distinguished from the S. hæmatobium. Leiper has recently noted a difference in that the male of S. mansoni has 7 testicles as against 4 for S. hæmatobium. With S. japonicum, the name of the Eastern species, there is not only the difference that the eggs are without spines, but, in addition, the skin of the adult parasite is not tuberculated, as is the case with the other two species. It is slightly smaller, the acetabulum projects more prominently, and the lower part of the male infolds more markedly than in S.

hæmatobium. Catto considers that the S. japonicum may live in both arteries and veins. The other two species only live in branches of the portal vein. The blood flukes are about 1/2 inch (13 mm.) long. All of these flukes live separately until maturity. At this time the female enters what is known as the gynæcophoric canal of the male; this canal is formed by the infolding of the sides of the flat male fluke, thus giving a rounded appearance to the male. The female is longer than the male (about 5/6 of an inch long), and is thread-like and of a darker color. Her two extremities project from the canal of the male in which she lives.

The oral sucker of the male is infundibuliform and is smaller than the pedunculated acetabulum. In the female the oral sucker is larger than the acetabulum. The eggs are fusiform, yellowish in color, have a thin shell and a terminal spine.

The most prominent symptoms of the Bilharz disease are hæmaturia and bladder irritation; later on calculus formation. In rectal bilharziasis the symptoms are more those of bleeding piles or of a mild dysentery.

There may also be involvement of the appendix. In the Japanese infection the symptoms point more to liver and spleen, there being ascites, cachexia, and a bloody diarrhœa.

The eggs of the S. japonicum are readily found in the fæces; they are about 100 × 70μ. They are oval, transparent, and with a smooth shell, within which can be made out the outlines of an embryo. Upon adding water the ciliated embryo begins to show movement in about ten minutes and shortly afterward bursts out of the shell and swims about actively. It is more melon-shaped than the miracidium of S. hæmatobium.

The life history is not known of any of these flukes. Looss conjectures that it is probable that the miracidium enters the skin, not requiring an intermediary host. Frequent experiments have failed to show any mollusk, etc., which attracted the embryo. Evidence seems to show that those who are constantly wading about in the water of the pools or the mud of the fields are the ones most subjected to infection.

Katsurada, by experiments with a cat and dog, has proved that infection will take place through the shaved skin of an animal held in infected water—none of the water being allowed to enter by mouth. Fully developed miracidia and male and female flukes were found in the portal vein. It is thought that further development of the miracidia in the body may account for the heavy infection.

Turner has recently noted the frequency of bilharzial affections of the lungs in South Africa (50% in natives) and he thinks this may be an important factor in prevalence of lung diseases in the natives of this region. He considers bathing in contaminated waters of prime importance in the causation of the infection which he thinks is probably by way of the skin.

A recent view is that the miracidium enters while bathing by the preputial channel, hence the value of circumcision.

If urine containing eggs is diluted with water the miracidium breaks out of the shell and swims about as if in search of some desired object.

The view is also entertained that the miracidium may gain access to the body through the drinking water; there is much evidence against this. However access

to the body is gained, it is known that the larval forms make their way to the liver where they develop. Arriving at maturity, the males and females become united and proceed to the terminal branches of the portal vein, where the irritating eggs, given off by the female, give rise to the symptoms.

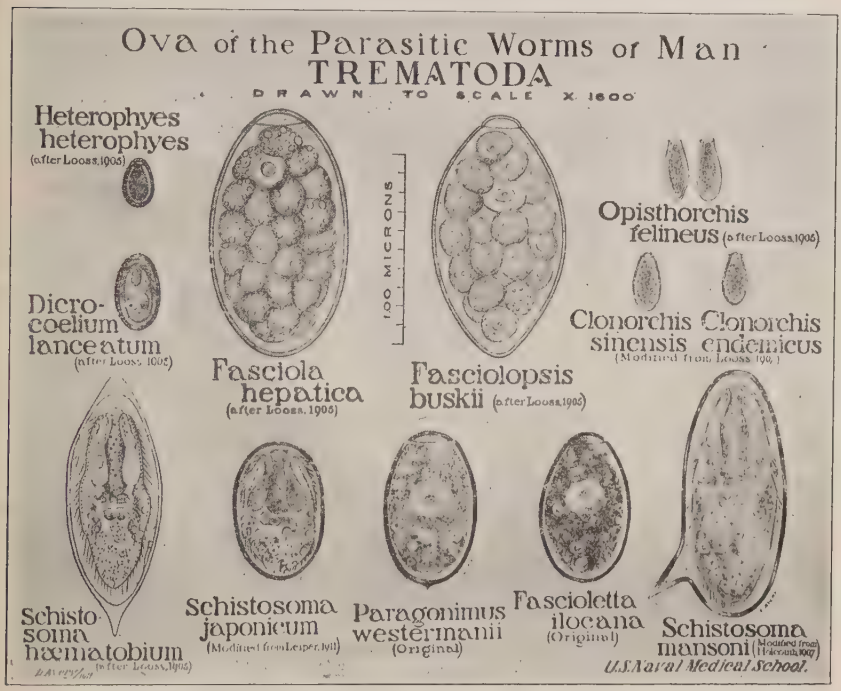


FIG. 66.—Trematode ova.

CESTODE OR TAPE-WORM INFECTIONS.

The cestodes and trematodes constitute the two great divisions of the flat worms. Anatomically, a tape-worm may be considered as a series of individual flukes united in one ribbon-like colony. The cestode segments, or proglottides are covered by an elastic cuticle and in their interior usually contain striated elliptical bodies composed of calcium carbonate about 5 to 25μ according to the species in which they are found.

These calcareous bodies are characteristic of cestode tissue. They have been mistaken for coccidia. There is no mouth or alimentary canal in tape-worms, the segments absorbing their nourishment through the general surface.

A tape-worm is divided into the segment-producing controlling head and the series of segments or proglottides together known as the strobila. The head and neck together form the scolex. Tape-worm heads are provided with suctorial or hook-like organs, or both, to enable them to hold on to the intestinal mucosa.

The hooks when present on the anterior extremity of the head are carried by a protrusible structure called the rostellum.

The importance of the head is generally recognized by the well-known fact that the permanent evacuation of one of these parasites is only arrived at when the head as well as the segments is expelled. Otherwise, additional segments will be produced.

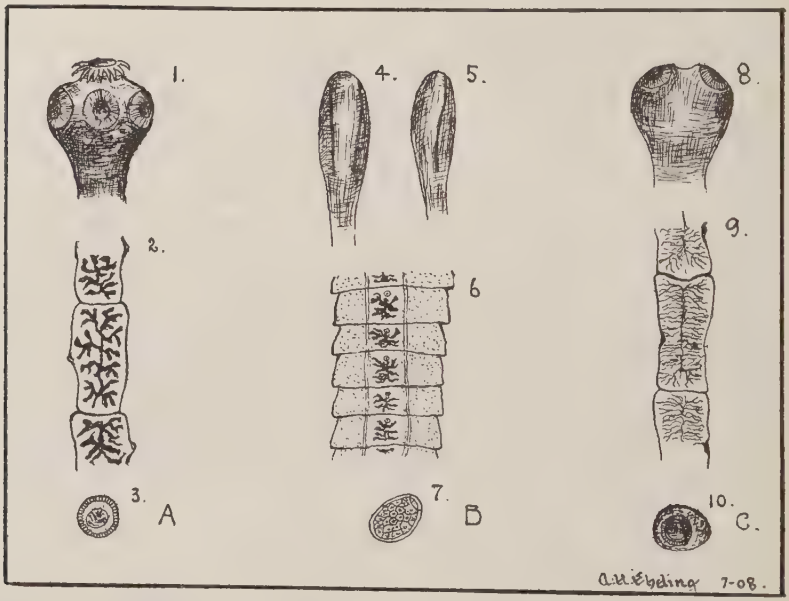


FIG. 67.—Tape-worms. A. 1, 2 and 3, Scolex, proglottides and ovum of Tænia solium; B. 4, 5, 6 and 7, Scolex, prologlottides and ovum of Dibothriocephalus latus; C. 8, 9, and 10, Scolex, proglottides and ovum of Tænia saginata. The onchosphere in 10 is shown within the outer yelk coating (frequently seen in stools). In 3 only the onchosphere within the embryonal shell is shown.

Even in tape-worms twenty-five to thirty feet in length, the head is no larger than a small shot. It carries the suckers or hooklets which best enable us to differentiate the different species. The segments adjacent to the head are immature—the sexually-mature ones being found from the middle of the body onward. The sexually-mature segment possesses a varying number of testicles: three in Hymenolepis nana and as many as 2000 in Tænia saginata. As with the flukes, they also have vasa deferentia, cirrus, ovaries, yolk glands, uterus, genital pore, etc. The

location of the genital pore and the character of the branching of the uterus are of the greatest importance in differentiation. The sexually-mature proglottides may either expel their ova, when these would be found in the fæces or, as is common, they break off and pass out themselves in the fæces. Then they either expel the eggs or may be eaten by some animal and in this way effect an entrance for their ova. It is an important practical point that the fæces of a patient with T. solium or T. saginata may not show any ova, these passing out in the intact segments. The oval operculated eggs of Dibothriocephalus latus, however, are constantly in the fæces.

The "hexacanth" or six-hooked embryo, also called the onchosphere, is the essential part of the egg. The embryonic envelope is dissolved off in the alimentary canal of the animal ingesting it, and the onchosphere bores its way through the gut to later become encysted in various tissues. In some tape-worms a ciliated embryo is liberated from the egg shell and swims about actively to enter some fish or other animal. When the six-hooked embryo reaches its proper tissue, the hooklets are discarded and a scolex similar to the parent one is developed. At this time we have a bladder-like structure with the scolex inverted in it. This is termed the proscolex stage. This little cyst with its scolex when ingested by another animal is digested, and the scolex, establishing itself in the intestine, develops a series of segments. The ciliated embryo of the D. latus does not form a cyst, but instead a worm-like creature similar to the adult. This is termed a Plerocercoid.

If the larval stage shows a single cyst and a single head, it is termed Cysticercus; if multiple cysts but only one head to each cyst, Cœnurus; while with multiple cysts and multiple heads in each cyst the term Echinococcus is used.

Where there is very little fluid in the cyst and the larva is of minute size, as with the Hymenolepis, the term Cercocystis is employed.

Key to Cestode Genera.

I. Head with two elongated slit-like suckers—Genital pores ventral—Rosette uterus. *Dibothriocephalidæ.*
  (*A*) Single set of genital organs in each segment. *Dibothriocephalus.*
  (*B*) Double set of genital organs in each segment. *Diplogonoporus.*
  (*C*) Immature forms showing characteristics of Dibothriocephalidæ—(collective group). *Sparganum.*

II. Head with four cup-like suckers; genital pores lateral. *Tæniidæ.*
  (*A*) Uterus with median stem and a varying number of lateral branches. *Tænia.*
  (*B*) Uterus without median stem and lateral branches.
    (1) Genital pores single. Rostellum with not more than two rows of hooks.
      (*a*) Suckers armed with numerous small hooklets. Fifteen to twenty testicles in each segment. *Davainea.*
      (*b*) Suckers not armed. Three testicles in each segment. *Hymenolepsis.*
    (2) Genital pores double. Rostellum with four or five rows of hooks. *Dipylidium.*

Tænia saginata (Tænia mediocanellata).—This very widely distributed tape-worm is often termed the unarmed tape-worm, to distinguish it from the T. solium or armed tape-worm.

It is from 10 to 25 feet long and has several hundred proglottides. The small pear-shaped head has four pigmented elliptical suckers and no hooklets. The segments are plumper than those of T. solium, hence the name saginata. The single lateral genital pore projects markedly and in a series of segments presents, as a rule, first on one side, and then on the opposite side of the next segment (alternating). The best way to distinguish a segment of the T. saginata from the T. solium is by counting the number of lateral uterine branches; these number fifteen to thirty, are quite delicate and branch dichotomously. The lateral divisions of the uterus of the T. solium are tree-like in their branching and only number five to twelve on each side.

T. solium has three ovaries while T. saginata has only two. The ox is the intermediate host. The eggs of Tænia have an oval outer shell which is filled with rather translucent, refractile yolk, often in globules. Within the oval shell is the more rounded cell of the six-hooked embryo with its thick striated membrane. The outer shell is often absent in the eggs found in the fæces, only the shell of the six-hooked embryo being found. The six-hooked embryo, having worked its way from the alimentary canal to the muscles or liver of the ox, becomes encysted (Cysticercus bovis). This little bladder-like structure is about 1/4 by 1/3 inches, and contains but a small amount of fluid. Being ingested by man's eating raw or imperfectly cooked meat, the adult stage becomes established in his alimentary canal.

It is probable that the various raw-meat cures have made the infection more common. In Abyssinia the infection is said to be universal, and a man without a tape-worm to be a freak. An important point is the fact that the larval stage almost never appears in man. It is this fact which makes it a so much less dangerous parasite than the T. solium, which readily establishes a larval existence in man if the ova are introduced into the human stomach. Cooking meat always destroys the cysticercus. A period of about two months elapses after the ingestion of the cysticercus before the mature segments pass out of the rectum. These not only make their exit with the fæces, but are also capable of wandering out at other times. In this they differ from the segments of T. solium. T. saginata next to Hymenolepis nana is the common tape-worm of the United States. Dr. Stiles has examined several hundred tape-worms in the United States during the past few years and has found only one T. solium.

Abnormalities of the scolex and proglottides are not uncommon with T. saginata. This is less frequently the case with T. solium.

Tænia solium.—The measly-pork tape-worm is smaller than the T. saginata and differs from it in having a globular head, with a rostellum which is crowned by twenty-six to twenty-eight hooklets.

In T. saginata a depression takes the place of the armed rostellum; the suckers

of T. saginata are, however, much more powerful than those of T. solium. The segments have only five to ten coarse branches and are expelled only at the time of defecation. The segments or the ova having been ingested by a hog, the six-hooked embryo is liberated and becomes encysted chiefly in the tongue, neck, and shoulder muscles of the hog, as an invaginated scolex. Pork containing this cysticercus (Cysticercus cellulosæ) is known as measly pork. This cysticercus contains much more fluid than that of the ox and is from 1/4 to 4/5 of an inch long. If one by chance should carry the egg on his fingers to his mouth, as the result of examining mature segments, the larval stage may be established in man. If this infection is not heavy, very few symptoms may be observed. The cysticercus, however, tends to invade the brain, next in frequency the eye, and so causes convulsions, death or blindness. Instead of only being the size of a pea, these cysts, when forming in the brain, may be the size of a walnut or larger. T. solium is comparatively common in North Germany, but is exceedingly rare in England and the United States.

Tænia africana.—This is an unarmed tape-worm, only about 5 feet long. It was found in a native soldier in German East Africa.

Garrison has reported from the Philippines a tape-worm with an unarmed rostellum, V-shape and spiral formation of the uterine stem with compact structure of the gravid uterus under the name of Tænia philippina. Another tape-worm, T. confusa of which only segments were found was reported by Ward from Nebraska.

Hymenolepis nana (Tænia nana).—This is generally known as the dwarf tape-worm—it is the smallest of the human tape-worms. It is from 1/4 of an inch to 1/2 inch in length, and is less than 1/25 of an inch in breadth. (10×1 m.m.)

The genus Hymenolepis has lateral genital pores, all of which are on the same side. These lateral genital pores cannot be made out in specimens as ordinarily examined. The head has four suckers and a rostellum, which is usually invaginated. The rostellum has a single row of twenty-four to thirty hooklets encircling it. Of the 150 to 200 narrow segments the terminal ones are packed with eggs which in the last two or three seem to fill entirely the disintegrating segments. It would seem that the fully mature segments disintegrate and in this way the eggs are set free in the surrounding intestinal contents.

The worms as found in fresh fæces after tæniacide treatment are frequently in an advanced state of disintegration so that it is impossible to make out the head or hooklets.

The eggs of this species are quite characteristic, there being two distinct membranes. The inner one has two distinct knobs, from which thread-like filaments proceed. The eggs of the H. diminuta have a thicker, striated, outer membrane and there are no filaments. The eggs of the Dipylidium caninum are similar, but are found in the fæces in aggregations—several eggs in a packet.

The dwarf tape-worm has been found to be the most common tape-worm in the United States. Dr. Stiles found it in about 5% of children in a Washington orphanage.

It has been estimated that in certain parts of Italy 10% of the children may be infected. The symptoms, expecially nervous ones, may be marked in this infection. It has been incriminated as a cause of chyluria. Although very small, yet the number of parasites may be very great, even more than 1000. In a case that I treated with thymol there were 1500 worms expelled. A form found in rats, which may be identical with H. nana, does not require an intermediate host. The six-hooked embryo bores into the intestinal villus and there develops a Cercocystis (larva of small dimensions with but little fluid). When fully developed, it drops into the lumen of the gut, and a new parasite is added to the already existing number of parasites. This expiains the heavy infection. H. diminuta and H. lanceolata have also been reported for man a few times.

H. diminuta is much larger than H. nana, being about 10 inches long. The suckers are small and the rostellum insignificant and unarmed. The intermediate host is some insect, as a moth; the definitive, the rat. As man is not liable to eat the insect hosts the infection is rare in man. Twelve cases have been reported for man of which 5 were from the U. S.

H. lanceolata is common in geese and ducks.

Dipylidium caninum (**Tænia cucumerina**) (**T. flavopunctata**).—This is a common parasite of dogs and cats. The larval stage is passed in lice and fleas. The cases of human infection have been principally in children, probably from getting dog lice or fleas in their mouths. The number of infections reported for man is about 40 and of these about 30 in children. The head has four suckers and a rostellum, which has three or four rows of encircling hooklets. The segments have the shape of melon seeds and have bilateral genital pores.

Davainea madagascariensis.—This tape-worm has been found in Siam and Mauritius. It is about 10 inches long. The head has four suckers and a rostellum with ninety hooklets. The suckers have rings of hooklets. The genital pores are unilateral. The cockroach is supposed to be the intermediate host.

There have been about 10 cases reported (Madagascar, Siam and British Guiana). There has also been reported a D. asiatica, the single specimen, however, lacking a head so that the exact genus is doubtful. It has been reported twice in children in Breslau. The intermediate host is thought to be a cyclops. Garrison reported cases from the Philippines.

Dibothriocephalidæ Infections.

Dibothriocephalus latus (**Bothriocephalus latus**).—This is frequently termed the broad Russian tape-worm. It has a small olive-shaped head with two deep winding suctorial grooves on each side; it has neither rostellum nor hooklets.

The segments are quite broad, being about 1/2 by 1/5 inch. At the end of the strobila they are more nearly square. The segments are very numerous, 3000 or more. The fully developed worm is about 30 feet long. The uterus in each segment is rosette-shaped and the genital pore is ventrally situated. The eggs of this species have an operculum and a ciliated embryo. This ciliated embryo swims around and either enters some fish, especially pike, directly or through an as yet unknown inter-

mediary. This parasite produces an intense anæmia similar to pernicious anæmia. It is a frequent parasite in Switzerland, Bavaria, Japan, Scandinavia, and Russia. Recently several cases have been reported from our Northwest, and some of the fish of the waters of that region are said to be infected. The larva is a pleroceroid and is about 1 inch long. It is said that salting, smoking, or other ordinary methods of preserving fish will not kill it.

A tape-worm, *Diplogonoporus grandis* has been reported from Japan. In this there are two complete sets of genital organs to each segment.

Somatic Tæniasis.

While rarely we may have the larval stage of T. solium present in man, and while certain bothriocephalid larvæ (Sparganum mansoni and Sparganum proliferum) infect man, yet they are unimportant as

Fig. 68.—Daughter cyst from hydatid cyst, considerably enlarged. (*Coplin.*)

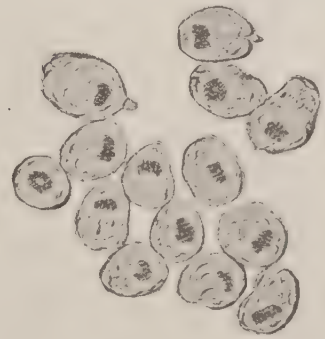


Fig. 69.—A group of daughter cysts from hydatid cysts. (*Coplin.*)

compared with the larval stage of the **Tænia echinococcus.** The adult stage of this parasite is passed in dogs. It is one of the smallest tape-worms known, being only about 1/6 inch long. It has a head with four suckers and a rostellum encircled with hooks. There are only three to four segments. The larval stage, on the contrary, gives one of the largest of larval cestodes. In man it may reach the size of a child's head. The larval stage is also found in hogs and sheep, and it is probable that by reason of the dog's eating the echinococcus cyst of such animals at the abattoir we owe the increase in this serious infection.

Man contracts the infection from association with dogs. The disease is peculiarly prevalent in Iceland. As stated above, the adult stage is passed in the intestine of the dog. Should the egg-bearing segments passed by the dog contaminate the hands

of man and a single egg be ingested, we may have hundreds of Tænia larvæ produced. The six-hooked embryo, leaving its shell, bores its way through the walls of the alimentary tract and especially seeks the liver, just as the embryo of T. solium seeks the brain and eye.

Griffith notes that in Australia from 10 to 15% of hydatid cysts occur in the lungs. The cyst wall is quite thin and the hydatid cachexia seems to appear earlier in the lung than in the liver cases.

In the development of the cyst, after the embryo has come to rest at some point in the liver, we have formed at first an indistinctly laminated external envelope

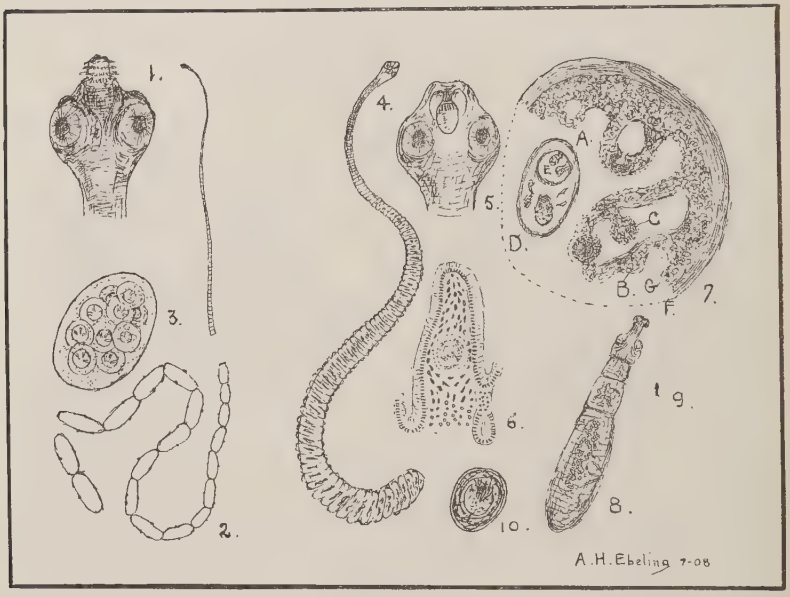


Fig. 70.—Tape-worms. 1, 2, and 3, Head, melon-shaped segments and egg packet of Dipylidium caninum; 4, 5, 6 and 10, entire worm magnified, head, larval stage in intestinal villus and ovum of Hymenolepis nana; 7, echinococcus cyst; A, mother cyst; D, daughter cyst; E, granddaughter cyst; C, scolex in brood capsule; B, brood capsule; G, parenchymatous layer; F, laminated layer; 8 and 9, Tænia echinococcus; 9, natural size.

with coarsely granular fluid contents. Later on the contents become transparent, and two distinct layers can be observed: 1. The external, markedly laminated one, and 2. the internal one, made up of small cells externally and large cells and calcareous corpuscles internally. This internal lining membrane is known as the parenchymatous or germinal layer. When the external layer is incised it curls up by reason of its elasticity. This is characteristic of such a cyst. In addition, we have an enveloping connective-tissue capsule formed by the surrounding liver substance. From the germinal layer arise the brood capsules and the scolices. In these brood

capsules we have the cellular layer external—just the reverse of the mother cyst. Scolices may develop either on the outside or inside of these brood capsules. It is interesting to note that one onchosphere may develop hundreds of scolices. From the parenchymatous layer of the mother cyst, daughter cysts are formed; these have an external stratified layer and an internal parenchymatous one; within them a varying number of scolices may develop. From these daughter cysts, granddaughter cysts may arise—all within the mother cyst—and hence are termed endogenous.

At times the daughter cysts work their way external to the mother cyst and proceed to develop in a manner similar to the endogenous formation. The exogenous

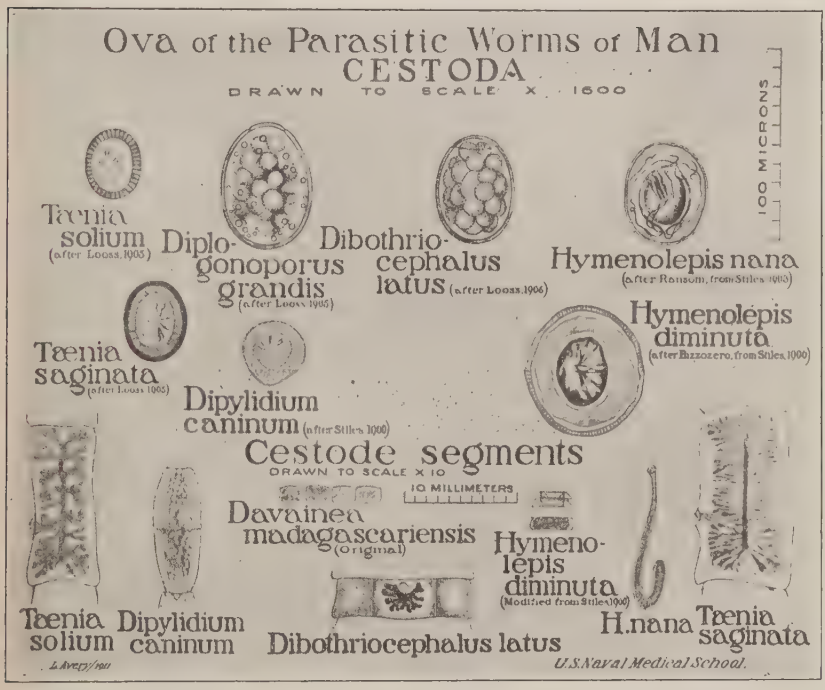


FIG. 71.—Cestode ova.

development is rare in man, but common in hogs. Hydatids containing no scolices are called sterile. These cysts may be as large as a child's head, but are usually smaller. The fluid of these cysts contains about 1% of NaCl, also a trace of sugar; in addition there is a toxin which produces urticaria and acts as a cardiac depressant. If any quantity should escape into the peritoneal cavity at operation, it may cause death. Hydatids develop very slowly, and the duration of the disease is usually from two to eight years.

Echinococcus multilocularis is possibly due to a species different from T. echinococcus. In this we have a honeycomb arrangement with cavities filled with a gela-

tinous material. The majority of these cysts are without scolices. This form of hydatid is very fatal.

Sparganum mansoni (**Bothriocephalus liguloides**).—This is a larval bothriocephalid which is about 5 to 10 inches long and has been reported ten times in Japan. It has been found in various parts of the body, as in pleural cavity, tissues about kidney, and in abscess of the thigh. They have been found in the urethra and under the conjunctiva. They resemble ribbon-like strings of fat.

Sparganum prolifer (**Plerocercoides prolifer**).—This has been reported from Japan as a larval form in the subcutaneous tissue. Stiles has found these larval forms in skin lesions in Florida. They show themselves as bizarre grub-like forms. They reproduce by budding.

CHAPTER XVIII.

THE ROUND WORMS.

CLASSIFICATION OF THE NEMATHELMINTHES (ROUND WORMS).

Class.	Family.	Genus.	Species.
Nematoda	Angiostomidæ	Strongyloides	S. stercoralis.
	Filariidæ	Dracunculus	D. medinensis
		Filaria	F. bancrofti
			F. loa
			F. perstans
			F. demarquayi
			F. ozzardi
			F. philippinensis
			F. volvulus
	Trichotrachelidæ	Trichuris	T. trichiura
		Trichinella	T. spiralis
	Strongylidæ	Eustrongylus	E. gigas
		Strongylus	S. apri
		Trichostrongylus	T. instabilis
		Triodontophorus	T. deminutus
		Œsophagostoma	O. brumpti
		Physaloptera	P. caucasica
		Ancylostoma	A. duodenale
		Necator	N. americanus
	Ascaridæ	Ascaris	A. lumbricoides
			A. canis
		Oxyuris	O. vermicularis
Acanthocephala		Gigantorhynchus	G. gigas
Hirudinea		Hirudo	H. medicinalis
		Limnatis	L. nilotica
		Hæmadipsa	H. ceylonica

NOTE.—The Strongyloides stercoralis was formerly described under two designations: (1) Anguillula intestinalis, a parasitic generation and (2) Anguillula stercoralis, a free living generation.

ROUND WORMS OR NEMATODES.

All nematodes are covered by a cuticle which varies in thickness, and is frequently ringed. The cuticle is moulted three or four times. The cuticle is formed by the underlying ectoderm which is, as a rule,

markedly developed in four ridges which divide the body into quadrants. Within the ectoderm is the body cavity, a space in which the reproductive organs lie in a clear fluid. The excretory system usually consists of two tubes which discharge near the head.

While the alimentary canal is more or less tube like in appearance it shows near the mouth a muscular œsophagus with a bulb-like expansion at the commencement of the remainder of the intestinal tract.

The testis and ovary are generally tube like. The sexes are, as a rule, separate. The male can usually be recognized by its smaller size, its curved or curled posterior end, and at times exhibiting an umbrella-like expansion—the copulatory bursa. The spicules, chitinous copulatory structures, may be observed drawn up in the worm or projected out of the cloaca. The genital opening of the female is ventral and usually about the mid-point; that of the male is close to the anus.

Certain papillæ in the region of the anus are valuable in differentiation. As a rule nematodes develop in damp earth from the eggs as rhabditiform larvæ. Very few nematodes are viviparous (Filaria, Trichinella).

The families Gnathostomidæ and Anguillulidæ are of very little importance in human parasitology. Gnathostoma siamense was once found in a breast tumor and Rhabditis pellio once in the urine.

Anguillula aceti, the vinegar eel, has been reported from the genito-urinary tract several times. Such cases can be explained by the prior contamination of the urine bottle or by the use on the part of the patient of a vinegar vaginal douche. The genera Rhabditis and Anguillula belong to the family Anguillulidæ.

A case of infection with a small nematode found in the papules of a skin infection, in a French boy is recorded as due to Rhabditis niellyi. The present view is that the parasites were embryos of A. duodenale, boring into the skin.

Angiostomidæ.

In this family we have heterogenesis.

Strongyloides stercoralis.—This parasite was formerly thought to be the cause of Cochin-China diarrhœa. It presents two generations: 1. Parasitical or intestinal form. 2. The free living or fæcal form.

1. The intestinal form (also known as Anguillula intestinalis) is represented only by females. These are about 1/12 of an inch (2 mm.) long and reproduce parthenogenetically. They have a pointed, four-lipped mouth, and a filariform œsophagus which extends along the anterior fourth of the body. The anus is situated near the sharpened posterior end, the vulva about the lower third of the body. The uterus contains a row of 8 to 10 elliptical eggs which stand out prominently in the posterior part of the body by reason of being almost as wide as the parent worm. They usually live deep in the mucosa and the embryos emerge from the ova laid in the

mucosa. The embryos escape from the eggs while still in the intestine, so that in the fæces we only find actively motile embryos. The eggs, which are strung out in a chain, never appear in the fæces except during purgation. As they greatly resemble hookworm eggs, this is a point of great practical importance. In fresh fæces we find hookworm eggs and Strongyloides embryos. The embryos are rather common in stools in the tropics. These embryos have pointed tails and are about 250×13μ. They have a double œsophageal bulb. They are about 250μ when they first emerge but may grow until they will approximate 500μ in the fæces. If the temperature is low, these rhabditiform embryos develop into filariform embryos, which being ingested form the infecting stage. It has been demonstrated that infection of man may also take place through the skin. If the temperature is warm, 25° to 35° C., these embryos develop into:

2. The free living form, Anguillula stercoralis. In this we have males and females, with double œsophageal bulbs, the male about 1/30 of an inch (3/4 mm.) long with an incurved tail and 2 spicules and the female about 1/25 inch (1 mm.) long with an attenuated tail; these copulate and we have produced rhabditiform larvæ, which later change to filariform ones. At this time the length is about 550 microns. These, being ingested, start up the parasitical generation. If these do not reach the intestine they die out.

Filariidæ.

This family is of the greatest importance to man. It is also one about which much confusion exists as to the adult type; hence anyone finding adult filariæ should fix them in hot 5% glycerine alcohol (alcohol 70%), and subsequently mount in glycerine gelatin. Formalin is not to be used, other than for a very brief period (2 to 6 hours) and then followed by the lacto-phenol method.

These worms are most likely to be seen as writhing thread-like worms, especially in the lymphatic glands and connective tissue, and about body cavities. They have a lipped or simple mouth and a filariform œsophagus. The male has an incurved tail with preanal and postanal papillæ which may be even corkscrew-like as in F. immitis. The spicules are unequal or there may be but one. The female is ovoviviparous, the vulva is at the anterior end and the uterus usually double.

Dracunculus medinensis (Filaria medinensis).—The Guinea or Medina worm, of which until recently only the female was known, is of great importance in parts of India, Africa, and Arabia. The female is a thread-like worm, about 20 to 30 inches long. The habitat is the subcutaneous and intermuscular connective tissue, especially of the lower extremity. It develops without symptoms. Finally a blister-like area appears on the surface of the leg, particularly about ankle-joint, which soon forms a painful ulcer. From this opening the

anterior end of the worm projects to pour forth its striated embryos upon contact with water.

The mouth is terminal and the body uniformly cylindrical. The uterus is a continuous tube filled with sharp-tailed, transversely striated embryos, 650×17μ, and constitutes the greater part of the body, the alimentary canal being pressed to one side. The genital organs probably discharge through the œsophagus. The body when being extracted is rather transparent. The tip of the tail is bent, forming a sort of anchoring hook. Recently Leiper fed monkeys on bananas containing in-

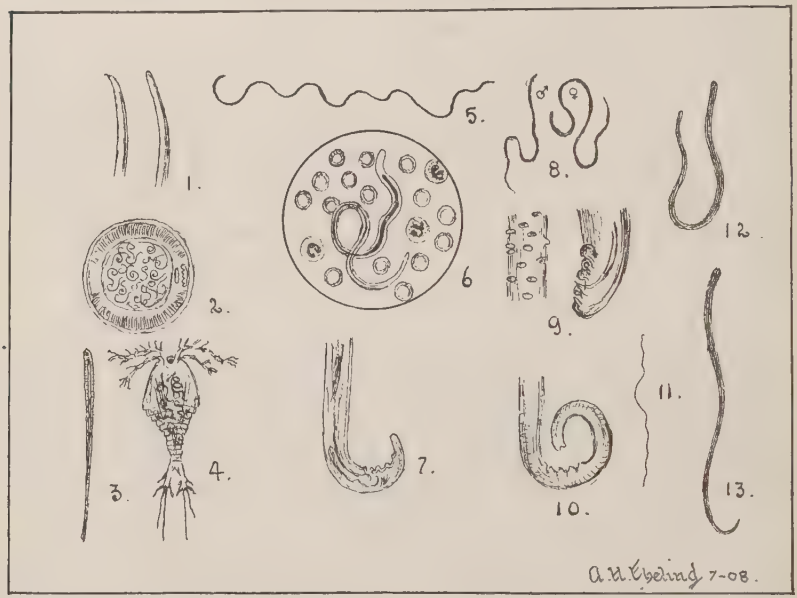


FIG. 72.—Round worms (Filariidæ). 1. Hooked posterior extremity and anterior extremity of Dracunculus medinensis; 2, cross section of uterus filled with embryos, D. medinensis; 3 and 4, free embryo and embryos of D. medinensis in intermediate host (Cyclops); 5, natural size of female Filaria bancrofti; 6, embryo of F. bancrofti in blood; 17, tail of male F. bancrofti; 8, male and female of F. loa (natural size); 9, tuberculated integument and posterior end of male F. loa; 10, posterior end of male F. perstans; 11, male of F. bancrofti (natural size); 12, blunt-tailed embryo of F. perstans; 13, sharp-tailed embryo of F. demarquayi.

fected Cyclops, and at the autopsy six months later obtained both male and female forms.

As regards the life history, Fedschenko, in 1870, showed that the embryos when liberated swam around in water and finally entered the bodies of species of the genus Cyclops. The female tends to come to the surface in the lower extremities, and experiments show that if on the blister-like points of emergence some water be squeezed out from a sponge, the uterus will eject a milky-looking fluid containing

myriads of embryos. This would indicate that the worm selects the lower extremity so that the embryos may gain access to the Cyclops when the host is wading through the water.

Leiper showed that a strength of HCl equal to that of gastric juice killed the Cyclops, but made the Dracunculus embryos very active. From this he judged that infection must probably take place from drinking water containing infected Cyclops. The suggestion of Leiper that wells harboring Cyclops be treated with steam, introduced by a pipe, seems to be valuable. The disease is known as "Dracontiasis."

Filaria loa (Filaria oculi).—This is a thread-like worm of West Africa about 1 to 2 inches long. The cuticle is characterized by distinct wart-like structures.

The anterior extremity is like a truncated cone with two papillæ at the base of the cone. The wart-like cuticular protuberances or bosses are about 12 to 15 microns in height. The females are 2 to 3 inches (50 to 70 mm.) long and about 1/2 mm. broad.

The males are smaller than the females and have three preanal papillæ and two postanal ones. There are two short unequal spicules. The life history is not satisfactorily established. The young are born ovoviviparously, and it has been suggested that the localized œdemas, known as Calabar swelling, may be due to the irritation produced by these eggs. These swellings are of hen's egg size, painless, do not pit on pressure and last about three days. They occur especially on the hands and arms. The embryos almost exactly resemble those of F. bancrofti. They have a diurnal periodicity, however, appearing in the blood about 8 A. M., increasing to noon and disappearing about 9 P. M. The adult worms have a tendency to wander about in the subcutaneous connective tissue, especially about the region of the orbit or even under the conjunctiva.

Adult worms of F. loa have been found and extracted, with an absence of the filarial embryos in the peripheral circulation of the patient. Leiper has just noted two species of Chrysops as intermediate hosts, the embryos developing in the salivary glands.

Filaria bancrofti (Filaria sanguinis hominis).—This is the most important of the filarial worms. It is a common infection in South China, India, the West Indies, and in the Pacific Islands, especially Samoa.

In medical books the embryos have been designated Filaria sanguinis hominis. This species is the cause of the common manifestations of filariasis, such as elephantiasis, varicose groin glands, chyluria, lymph scrotum, etc.

Filarial diseases are prone to lymphangitis attacks. Thus in lymph scrotum an erysipelatoid condition of the scrotum with high fever and chills may result. This condition is at times mistaken for malaria. Varicose groin glands may be mistaken for hernia. In the Philippines very few symptoms are noted in those affected with filariasis. Occasionally chylocele or chyluria is reported.

F. bancrofti lives in lymphatics of trunk and extremities. At times the fine white thread-like worms may be seen as writhing coils in lymphatic glands.

The sexes are usually found together. The females are about 3 inches long and the males less than 2 inches. The tails of both sexes are incurved, but that of the male is more so. The head is club-shaped. The vulva opens 1.2 mm. from the anterior end. There are 2 uterine tubules. The sheathed embryos are supposed to be born viviparously and Manson supposes that as a result of injury to the parent worm and resulting extrusion of eggs, the blocking of lymph channels occurs.

A very interesting fact is that people with elephantiasis fail to show larvæ in the peripheral circulation. Manson considers that it is due to the blocking of the lymph channels.

These embryos show a nocturnal periodicity. During the day they remain in the lungs, and larger arteries.

If the patient sleeps in the day time and is active at night the nocturnal periodicity or presence of embryos in peripheral circulation is inverted. In the case of F. loa, however, a change of habits does not change the periodicity of the filarial embryos, they continue to appear in the peripheral circulation by day even if the patient sleeps at that time.

The disease is transmitted especially by Culex fatigans. The sheathed embryos, getting into stomach of mosquito, wriggle out of the sheath, they then bore their way through walls of stomach and enter into a sort of passive stage, during which further development takes place. They finally become distributed in the muscles of the thorax and make their way along the fleshy labium, to enter the wound in a person bitten by a mosquito, by way of Dutton's membrane. This takes about twenty days at which time the larvæ are about 1/16 inch long and have an alimentary canal.

Filaria perstans.—The adults are found in connective tissue and deeper fat, especially about the mesentery and abdominal aorta.

The female is about 3 inches (75 mm.) long; the male is rarely found and is less than 2 inches long. These worms are characterized by incurved tails, the extremity of which has two triangular appendages giving a bifid appearance. The embryos do not possess a sheath and have a blunt tail. The life history is unknown. Both mosquito and tick have been incriminated. The embryos are always present in the peripheral circulation—hence perstans. There does not seem to be any symptomatology.

It is of historical interest that F. perstans was once considered the cause of sleeping sickness.

Filaria volvulus.—This is a rather common parasite of Central Africa. The male is about 1 1/2 inches (35 mm.) and the female about 5 inches long. The females are so interlaced in the fibro-cystic swellings that it is difficult to determine their length. The tumors start from the presence of a worm in a lymphatic. The tumors are easily enucleated. Adults are striated. They are found in cystic tumors, especially about the axilla and popliteal space. The cystic contents contain abundant sheathless larvæ about 300μ long; they are not found in the peripheral circulation. Life history unknown, although it has been suggested that a species of Glossina may be concerned.

Filaria demarquayi.—The habitat of this filarial worm is the West Indies. The embryo has no sheath and has a sharp tail. Other filarial species which have

been reported are F. magalhæsi, F. ozzardi, F. volvulus, F. powelli, and F. philippinensis. A species called F. gigas is now considered to have been only the hair of the leg of a fly. The embryos have usually been given such names as F. nocturna, F. diurna, etc. Of course the embryos and the parent should have the same name. It has been proposed to designate these embryos the same as the parent, but with the use of the term Microfilaria instead of Filaria.

The points usually noted in the description of filarial embryos are:

1. Presence or absence of periodicity of embryos in peripheral circulation.
2. Presence or absence of a sac sheath around the embryo.
3. Accurate measurements.
4. Shape and description of head and tail ends.
5. Character of movement.
6. Location of V spot and break in cell column in stained specimens.

Key to Filarial Larvæ.

A. Sheath present.

1. No periodicity.
   F. philippinensis. Tightly-fitting sheath; not flattened out beyond extremities. Tail is pointed and abruptly attenuated. Lashing progression movement. 320×6.5μ.
2. Periodicity exhibited.
   a. Nocturnal periodicity.
      F. bancrofti (F. nocturna). Pointed tail; loose sheath; lashing movement. 300×7.5μ. V spot 90μ from head; break in cells 50μ from head.
   b. Diurnal periodicity.
      F. loa (F. diurna). Pointed tail; loose sheath; 245 by 7 microns. V spot 60 to 70 microns from head, break in cells 40 microns from head.

B. *Absence of sheath.* None of these exhibit a periodicity, being continuously present.

1. Blunt tail—F. perstans. 200×4.5μ.
2. Sharply-pointed tail:
   a. F. demarquayi. 210×5μ.
   b. F. ozzardi. 215×5μ.

Note.—A filarial embryo, F. powelli, reported once. It has a sheath, nocturnal periodicity, and is about 130×5μ.

Trichotrachelidæ.

These have a long thin neck and a thicker terminal portion. The œsophagus is of the single row of cells type. The anus is terminal; there is only one ovary.

Trichuris trichiura (**Trichocephalus dispar**).—This is usually called

the whip-worm—the thickened body representing the handle and the narrow neck the lash. It is one of the most common parasites in both temperate and tropical climates.

The egg is very characteristic in having an oval shape with knobs at either extremity. It resembles a platter with handles. The male is almost 2 inches long, and has the terminal portion curled up in a spiral. It has a single terminal spicule.

The female is a little longer than the male, and has the terminal part in the shape of a comma instead of being coiled. The neck only contains the œsophagus which

Fig. 73.—Round worms. 1, Encysted embryo of Trichinella spiralis; 2, male and female of T. spiralis; 3, male and female of Trichocephalus trichiurus; 4, egg of T. trichiurus; 5 and 6, head and male and female of Ascaris canis; 7, 8, and 9, head, egg and male and female of Oxyuris vermicularis; 10, 11 and 12, head, egg and tail of Ascaris lumbricoides; 13 and 14, head and egg of Echinorhynchus gigas; 15, 16 and 17, parthenogenetic female and rhabditiform and filariform embryos of Strongyloides stercoralis.

is contained in a groove in large cells which form a single row like a string of pearls. These cells play a digestion rôle. The vulva opens at the upper end of the thickened terminal end which contains an intestine lying between the ovary and uterus. The great powers of resistance of the ova may account for their general distribution; they may live for months under conditions of freezing and so forth. There is no intermediate host. The worm arrives at sexual maturity in about one month after ingestion. The whip-worm prefers the cæcum, but also lives in the lower end of the ileum and the appendix.

The neck burrows into the mucosa, and much importance has been attributed by the French to the possibility of this paving a way for the entrance of pathogenic bacteria. They do not seem to produce serious symptoms.

Trichinella spiralis (Trichina spiralis).—The cause of trichinosis is usually termed Trichina spiralis in medical works.

The adults live in the duodenum and jejunum; the males are about 1/16 of an inch (1.5 mm.) long with two tongue-like caudal appendages and without a spicule. These two lateral projections enable the male to hold the female in copulation—the cloaca being evaginated to act as a penis.

The females are about 1/7 of an inch (0.3 to 0.4 mm.) long. The female gives off embryos from the vulva which is near the mouth end (viviparous).

These parasites can be seen with an ordinary magnifying glass. With higher

FIG. 74.—Trichina spiralis (*Ziegler*).

powers the œsophagus has the appearance of a serrated line instead of an œsophageal bulb. The male is about 40μ broad and has a prominent testicular enlargement filling the posterior extremity. The female is about 60μ broad and has a rounded posterior extremity with a prominent slit-like cloaca. It is in this posterior extremity that the female increases in size as she becomes filled with eggs. The vulva is in the anterior third. After fertilization of the females the males die, and the females bore into the intestinal mucosa and begin to produce embryos to the number of more than 1000 each. These gain access to the lymph channels and are distributed by the blood stream to the striated muscles. Embryos reaching other tissues fail to develop.

It is about ten days before they reach the muscle. In the muscle they become encysted as the oval lemon-shaped areas containing coiled-up embryos that everyone is familiar with. These oval areas are about 450×250μ and have a chitinous capsule.

The encysted trichinæ are found chiefly in the muscle fibers of the tongue and

diaphragm and may remain alive as long as ten to twenty years; finally, however, the cyst undergoes calcareous infiltration and the embryo dies.

When uncoiled the embryo is about 1 mm. long with the mouth at the attenuated end. Among cannibals it would be easy to keep the cycle going by eating improperly cooked or raw human meat, the parasite being thus transmitted.

As this would not explain the transmission among civilized men, the following is the life history: Man obtains his infection from eating raw pork, the embryos encysted in the muscle of the hog being liberated in the stomach, and the males and females developing in the intestine as above described. The hog may gain his infection by eating the meat of other hogs or rats. These rats eat scraps of pork at slaughter houses and become infected. Being cannibals, rats when once infected, continue to propagate the infection. In man, during the first two or three days while the adults are breeding in the intestine, we have gastrointestinal symptoms.

It is during this period or at any rate before the fifth day that purging may be of benefit. About ten to twenty days after infection the embryos begin to wander and we have the acute muscle pains. In the diagnosis we should try to obtain specimens of the pork which has caused the trouble in order to examine for encysted trichinæ, or to feed to white rats or rabbits, subsequently examining the diaphragm of these animals for encysted trichinæ or the intestine for adult trichinæ. Excision of a small piece of the deltoid of man may confirm the diagnosis. The best method is to take blood in 3% acetic acid, centrifuge, and examine for larvæ.

During the diarrhœal stage we may examine the stools for adult worms, in particular dead males or possibly actively motile embryos—these latter are about $90 \times 6\mu$.

Always examine the blood for eosinophilia.

It is well to remember that the parts of meat which trichinæ prefer (muscle of diaphragm, of neck, etc.) are often used in sausage. Unfortunately it is almost impossible to detect the embryos in sausage meat.

Strongylidæ.

In this family the male has a caudal bursa, a prehensile sort of expansion at the posterior end for copulatory purposes.

The mouth is usually provided with six papillæ and at times with a chitinous armature. Those without the chitinous armature are included in the subfamily Strongylinæ (Strongylus, Trichostrongylus)

while those having an armed mouth are in the subfamily Sclerostominæ (Ancylostoma, Necator, Triodontophorus, Œsophagostoma, Physaloptera).

Eustrongylus gigas (Strongylus renalis).—This is the largest round worm infecting man; it is usually found in the pelvis of the kidney (giant strongyle).

Two or more worms may so distend the kidney as to convert it into a mere shell. Pain, hæmaturia and other symptoms of pyuria, together with the finding of the eggs, make the diagnosis. There seem to be seven authentic and eight doubtful cases of infection in man.

The females are about 40 inches (1 m.) long and about 1/3 of an inch (8 mm.) in breadth while the male is about 10 inches (25 cm.) long.

The collar-like copulatory bursa of the male distinguishes it from Ascaris as does also the dark red color. The source of infection is unknown but it has been suggested that the larval stage may exist in fish.

Many of the reported cases were simply fibrinous clots from ureters or wandering round worms.

The very characteristic ova, with gouged-out oval depressions, may be found in the urine, and are diagnostically confirmatory.

Strongylus apri.—This nematode is common in the lungs of hogs, producing a bronchitis in young animals but apparently harmless for adult ones. It has been reported once from the lungs of a six-year-old boy. The male is about 1 inch (25 mm.) long with two long spicules. The female is about 2 inches long and has a sharply hooked posterior extremity with the vulva just beyond the bend. The mouth has six lips. The eggs contain embryos when laid.

Trichostrongylus instabilis.—This is a small strongyle formerly known as Strongylus subtilis. The male is about 1/6 of an inch (4 mm.) long, and the female about 1/4 of an inch (6 mm.). Anteriorly it tapers to a pointed head end which is only about one-tenth the thickness of the posterior extremity. The male has a bursa and two prominent equal spicules. It has been found in the upper part of the small intestine of inhabitants of Egypt and Japan. It does not appear to produce symptoms. Ova like hookworm ones ($63 \times 41 \mu$).

Triodontophorus deminutus.—This is a small round worm with three forked teeth taking origin from the pharyngeal lobes. The collar-like mouth orifice is made up of 22 rounded plates just inside the round mouth opening. They are less than 1/2 inch long and have once been found in the intestinal canal.

Œsophagostoma brumpti.—Six young females were found in a cyst of the colon in an African negro. They were about 1/3 inch (8 mm.) long. The anterior end presents an ovoid protuberance with a second cuticular inflation just below it. The buccal capsule is very shallow and surrounded by about a dozen chitinous plates. The mouth has six papillæ.

This species has recently been reported by Thomas in a native of Brazil.

Physaloptera caucasica.—Mouth with two equal laterally placed lips, each having three papillæ and three teeth. The male has a lancet-shaped posterior extremity and is about 1/2 inch long (14 mm. by 0.71 mm.). Female is about 1 inch long

(27 mm.) with a rounded tail end. Found only once in the alimentary canal of a native in the Caucasus. Leiper has recently reported a species P. mordens from Uganda, one case.

Ancylostoma duodenale (Dochmius duodenalis.)—The hookworm, so called from the hook-like appearance of the ribs of the copulatory bursa or from the hook-like projection of the head dorsally, is probably the most important of the parasitic worms. This species in Europe and Africa and the Necator americanus in the New World cause an immense amount of invaliding. The Egyptian anæmia and the Porto Rican anæmia are caused by this parasite.

Goeze found a hookworm in a badger in 1782. He named the parasite Ascaris criniformis. Froelich, in 1789, found hookworms in the fox and called them hookworms from the hook-like ribs of the copulatory bursa. He proposed the generic name Uncinaria. Therefore Uncinaria belongs to the hookworms of the fox and is not valid for any human species.

In 1838, Dubini found a hookworm as a human parasite. On account of the four ventral teeth projecting from the mouth he gave it the name Agchylostoma or correctly Ancylostoma.

Bilharz and Griesinger noted the connection of the parasite with Egyptian chlorosis, but it was not until the time of the St. Gothard tunnel (1880), that the importance of the parasite was recognized. Grassi noted the diagnostic value of the ova in fæces in 1878. In 1902, Stiles noted and described the hookworm found in the United States as different and proposed the name Uncinaria americana, later changed to Necator americanus. A. J. Smith had also recognized the morphological differences.

Hookworms may be found in the small intestine (jejunum) of man in enormous numbers. They either produce their effects by feeding on the mucosa or by causing loss of blood.

The males are little more than 1/3 of an inch (9 mm.) long and the females little more than 1/2 inch (13 mm.) in length. The male can readily be distinguished by his umbrella-like expansion or copulatory bursa. The tail of the female is pointed. The vulva of A. duodenale is located in lower half of the ventral surface; that of N. americanus in upper half. The large oval mouth of the Old World hookworm has four claw-like teeth on the ventral side of the buccal cavity and two on the dorsal aspect. In N. americanus the buccal capsule is round, smaller and the ventral teeth are replaced by chitinous plates. Dorsally there are two similar but only slightly developed lips or plates. A very prominent conical dorsal median tooth projects into the buccal cavity. Through it passes the duct of the dorsal œsophageal gland. The copulatory bursa of the N. americanus is also different, being terminally bipartite and deeply cleft in the division of dorsal ray rather than tripartite and shallow as with the A. duodenale.

The delicate-shelled eggs pass out in the fæces, and in one or two days a rhabditiform embryo ($200 \times 14\mu$) is produced.

The mouth cavity of the embryo is about as deep as the diameter of the embryo at the posterior end of the mouth cavity; that of Strongyloides is only about one-half as deep as the diameter.

A temperature of 1° C. kills the eggs in twenty-four to forty-eight hours. After moulting twice, it remains rather quiescent but still lying inside the discarded skin. It reaches this stage in from four to fourteen days according to the temperature.

The soil in the area of the hookworm-egg-laden stool becomes infested with these

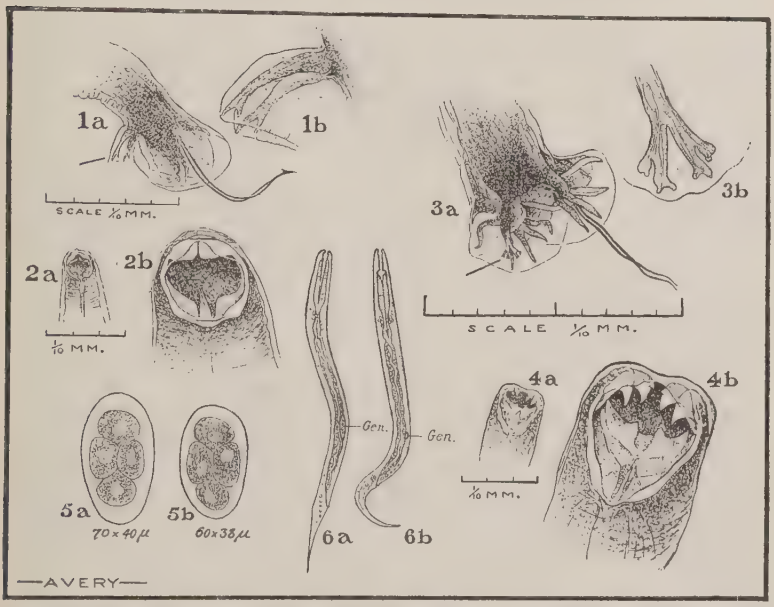


FIG. 75.—1a, Copulatory bursa of *Necator americanus*, showing the deep cleft dividing the branches of the dorsal ray and the bipartite tips of the branches; also showing the fusion of the spicules to terminate in a single barb. Scale 1/10 mm. 1b, Branches of dorsal ray magnified. 2a, The buccal capsule of *N. americanus*. 2b, The same magnified. 3a, Copulatory bursa of *Ancylostoma duodenale*, showing shallow clefts between branches of the dorsal ray and the tridigitate terminations. Spicules hair-like. 3b, The dorsal ray magnified. 4a, The buccal capsule of *A. duodenale*, showing the much larger mouth opening and the prominent hook-like ventral teeth. 4b, the same magnified. 5a, Egg of *N. americanus*. 5b, Egg of *A. duodenale*. 6a, Rhabditiform larva of Strongyloides as seen in fresh fæces. 6b, Rhabditiform larva of hookworm in fæces eight to twelve hours after passage of stool.

larvæ which will even climb up blades of grass. It is for this reason that children with their bare feet are so liable to infection. (If the larvæ get into water they sink to the bottom.) It is at this stage that it burrows into the skin of man, producing the so-called "ground-itch" at the site of entrance. Having gained access to the lymphatics and veins, they eventually reach the lungs. Here they get into the bronchioles and

undergo a third moulting. They then work their way up the trachea to the glottis and are swallowed to then become adults in the intestine. Dr. Stiles, while accepting this theory of the life history, thinks it probable that infection is also brought about by swallowing directly some infecting stage.

Very young dogs can be infected with human hookworm larvæ, but infection of man with the dog hookworm (A. caninum) has not been reported.

The infecting stage is not a young larva but one in which the cuticle of a former larval stage instead of being cast off remains and acts as a protecting sheath for the more mature larva within. In this stage larvæ may remain alive for six to twelve months and have greater powers of resistance than younger larvæ. Introduction, either by skin or mouth, of these cuticle-covered larvæ is followed by finding of eggs in the fæces in about fifty days.

It has been claimed that where ordinary microscopical examination for ova will show 40% of infections and methods involving concentration 55% that cultural methods will show 99%. A convenient method of culturing is to make a pile of filter-paper circles of 2 inches diameter and about 1/4 inch high and place in the center of a 4-inch Petri dish. Fill the dish with water about to the height of the filter-paper and spread a thick layer of fæces on the top of the filter-paper island. The larvæ hatch out in about six days and swim out into the clear surrounding water. They are best found by centrifuging the fluid containing them.

Of the three standard drug treatments that of thymol seems to be preferable to betanaphthol and vastly so to eucalyptus oil. In giving thymol it is imperative that neither alcohol in any form nor fats in any form be given on the day of treatment. Stiles prefers to divide his thymol into three doses, 1/3 at 6 A.M., 1/3 at 7 and 1/3 at 8 followed by epsom salts at 10 A. M. The patient should be on a restricted diet and be given two doses of salts on the two days preceding the administration of thymol.

Necator americanus.—This is the species of hookworm found in the southern states of the United States and the West Indies.

It is very prevalent in Guam, L. I. It was found by Looss in pigmies from Central Africa, so that this parasite was undoubtedly brought to America by slaves. It is not rare in Ceylon, India and the Philippine Islands.

The copulatory bursa of this parasite has double spicules which fuse terminally in a barb, while A. duodenale has two fine hair-like spicules. The head of Necator has a more marked dorsal bend than Ancylostoma.

To sum up the differences between this species and A. duodenale we have with Necator: 1. Smaller oral cavity which shows rib-like projections leading to the two ventral plates instead of the four prominent projecting teeth. 2. The dorso-median ray of the caudal bursa is deeply cleft and shows bipartite divisions terminally instead of having

a shallow cleft and tripartite division. 3. The vulva of the female is placed in the anterior third.

The eggs of N. americanus are larger than those of A. duodenale. In hookworm disease we have ground itch, tibial ulcer, anæmia, interference with physical and mental development and, in bad cases, dirt eating.

Shade, moisture and sandy soil seem essential factors for the development of hookworm. Prophylaxis is essentially one connected with soil pollution. The

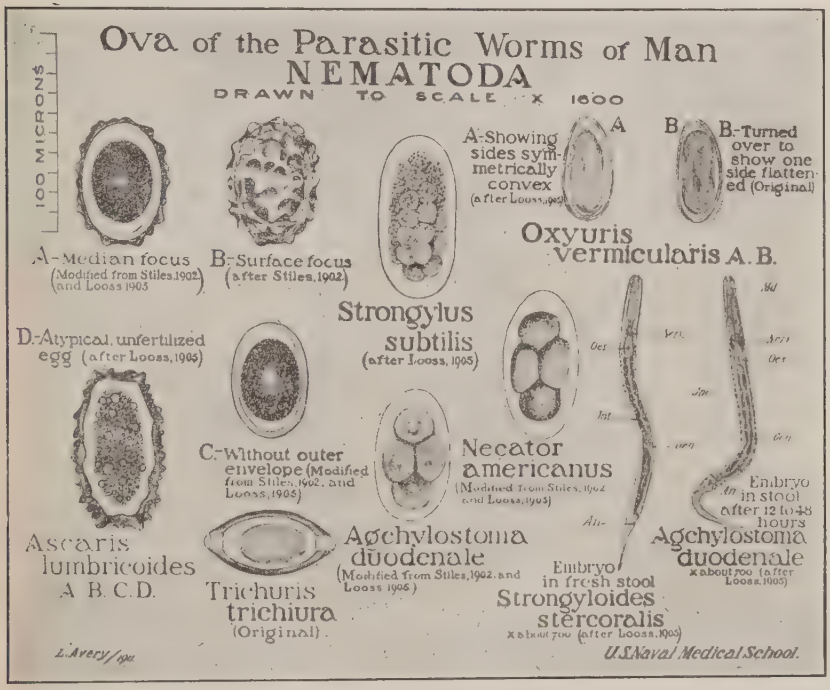

Ova of the Parasitic Worms of Man
NEMATODA
DRAWN TO SCALE X 1600
100 MICRONS
A-Showing sides symmetrically convex (after Looss,1905)
A
B
B-Turned over to show one side flattened (Original)
Oxyuris vermicularis A.B.
A-Median focus (Modified from Stiles,1902 and Looss 1905)
B-Surface focus (after Stiles,1902)
Strongylus subtilis (after Looss, 1905)
D-Atypical, unfertilized egg (after Looss,1905)
C-Without outer envelope (Modified from Stiles,1902, and Looss,1905)
Necator americanus (Modified from Stiles,1902 and Looss,1905)
Embryo in stool after 12 to 48 hours
Ascaris lumbricoides A.B.C.D.
Trichuris trichiura (Original)
Agchylostoma duodenale (Modified from Stiles,1902, and Looss 1905)
Embryo in fresh stool
Strongyloides stercoralis x about 700 (after Looss,1905)
Agchylostoma duodenale x about 700 (after Looss,1905)
L.Avery/1911
U.S.Naval Medical School.

FIG. 76.—Nematode ova.

subjection of the fæces to some septic tank process is more reliable than chemical disinfection or burying the fæces 8 to 10 inches under ground.

ASCARIDÆ.

These have three papillæ around oral cavity, one dorsal and two ventral. The male has two equal-length spicules. An intermediary host is not needed in the life history of this family.

Ascaris Lumbricoides.—The male round or eel worm is from 5 to

8 inches (18 cm.) long and the female from 7 to 15 inches (30 cm.) in length. They are from 1/7 to 1/4 of an inch (5 mm.) in diameter.

It is probably the most common parasite of man, especially in children and as it does not require an intermediate host infection takes place through food or drink or by fingers of children who have been playing where soil pollution exists.

The normal habitat is the upper part of the small intestine, hence the ease with which they are vomited up. The three papillæ-like lips with a constriction just behind are easily studied with a hand glass. The very long, whitish, convoluted, thread-like tubes of the uterus lead to the opening of the vulva anteriorly and ventrally. The male has two large lance-like spicules.

The body of the worm is transversely striated and resembles the ordinary earthworm, but is more grayish than red. The ova are very characteristic with a rough mammillated exterior. This at times is shelled off and we have a smooth egg which may be mistaken for eggs of other parasites. The eggs leave the body in the fæces

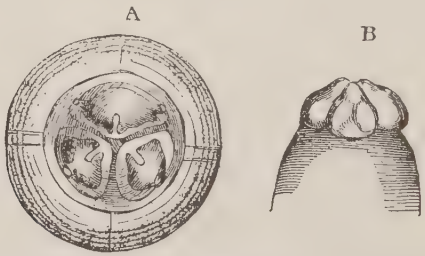


FIG. 77.—Anterior extremity of *Ascaris lumbricoides; A*, seen from front; *B*, seen from dorsal surface. (*Tyson after Railliet.*)

and after a long time —a few weeks to several months, according to temperature—develop an embryo which remains in the shell until swallowed by man. It is stated that they will remain alive for years. On being swallowed, the embryo leaves the egg and we have males and females developing in the small intestine. In countries where such parasites abound, as in Guam and the Philippines, the possibility of their getting into the peritoneal cavity through operative measures on the intestine must always be thought of.

Guiart considers it probable that Ascaris may suck blood, produce intestinal ulcerations and bacterial infections, and perforate intestine. Their entrance into bile ducts or into larynx (vomited) must be considered.

At autopsy they may be found perforating the appendix or even filling up the pancreatic duct.

Some think that the symptoms of itching of nose and anus, vertigo, or convulsions and anæmia may be due to a toxin secreted by the worm.

Ascaris Canis.—This is a parasite of the dog and cat, but is occasionally found in children. It is much smaller than the A. lumbricoides—male is 2 to 3 inches long,

female 4 to 5 inches in length. The parasites are characterized by the presence of wing-like projections from the anterior end (arrow-like head).

Oxyuris vermicularis.—This parasite is also known as the pin-worm or seat-worm and is more frequent in children than in adults.

The male is about 1/6 of an inch long and the female a little less than 1/2 inch in length. The male has an incurved tail with a single spicule and the female a long tapering tail. The vulva is in the upper third.

These worms have a clear slightly bulbous, pipe mouth-piece-like projection surrounding the three-lipped anterior extremity. There is a well-marked bulb œsophagus.

The eggs are thin-shelled plano-convex, and show a coiled-up embryo. After ingestion of eggs, the adults develop in the small intestine where copulation takes place; the males then die. The fertilized females go to the cæcum and colon where they remain until they reach maturity. At this time the females wander to the rectum where they either expel their ova or themselves work their way out of the anus. This usually occurs at night, and the scratching induced by the itching causes the eggs to be widely spread about the region of the anus. The worms may also wander into the vagina, urethra, or under prepuce. It will be seen that as a result of the scratching, the fingers become contaminated with ova which may be carried to the mouth and so cause a fresh infection, no intermediate host being required. The examination of the material under the finger nails of children harboring this parasite may show eggs under the microscope. A knowledge of the life history—the early location in the small intestine, and later on in the large—shows that treatment should be dual in its direction—enemata for the gravid female in the rectum and santonin and calomel for the young adults in the small intestine.

The diagnosis is preferably made by examining the stools for the white, thread-like females which are expelled after a diagnostic dose of calomel and salts, rather than by searching for the eggs.

These females, which are packed with embryo containing eggs, may be seen wriggling on the surface of the freshly passed fæces. In handling these worms one should be careful as they are apt to cause infection should the eggs get on the fingers.

Acanthocephala.

These are called thorn-headed worms on account of a proboscis which projects anteriorly like a little peg.

There are several rows of hooks surrounding this projection which are directed backward to enable the parasite to attach itself to the intestinal wall. The worm absorbs nourishment through the general body wall, there being no alimentary canal or mouth. These worms are common in hogs. The three-shelled eggs are very striking and the intermediate stage is in June bugs.

The Echinorhynchus or Gigantorhynchus gigas.—This parasite is about 6 inches (15 cm.) long for the male and 10 to 12 inches (25 cm.) for the female. It has

transverse rings and resembles Ascaris but is more white in color. It is said to be not uncommon in southern Russia.

The Echinorhynchus or Gigantorhynchus moniliformis might be contracted by persons eating death-watch beetles as is sometimes done for the improvement of the complexion.

Hirudinea (Leeches).

Hirudo medicinalis.—This is the leech used medicinally for the abstraction of blood. They have a secretion which prevents coagulation of the blood so that when they are removed the wound still continues to bleed.

Limnatis nilotica.—This species has been found in many parts of Northern Africa and, gaining access to the stomach through drinking-water, it wanders to the pharynx, nares, and even trachea. Manson refers to a case of obstinate epistaxis and headache caused by a leech in the nostril.

This leech is about 4 inches long (8 to 10 cm.) and about 1/2 inch (1.2 cm.) broad. The dorsal surface is greenish brown with narrow orange brown borders. The young leeches are only about 1/8 inch (3 mm.) long and taken in with the drinking water may attach themselves to the surface of some mucous membrane and after some weeks reach adult size.

Hæmadipsa ceylonica.—These are land leeches found in India, Philippines, Australia, and South America. They are only about 1 inch (25 mm.) long and are slender. They leave the damp earth to climb shrubs and from there to drop on animals or man passing through the forest. The bites are painless, but may be followed by ulcers. They may get into the nostrils.

They will even penetrate thick clothing in order to reach the skin.

CHAPTER XIX.

THE ARACHNOIDS.

Classification of the Arachnoidea.

Order.	Family.	Subfamily.	Genus.	Species.
Acarina	Trombidiidæ		Trombidium	T. holosericeum
	Gamasidæ		Dermanyssus	D. gallinæ
	Tyroglyphidæ		Tyroglyphus	T. farinæ
				T. longior
	Sarcoptidæ		Sarcoptes	S. scabiei
	Demodicidæ		Demodex	D. folliculorum
	Tarsonemidæ		Pediculoides	P. ventricosus
	Ixodidæ	Argasinæ	Argas	A. persicus
				A. miniatus
			Ornithodoros	O. savignyi
		Ixodinæ	Ixodes	I. ricinus
			Hyalomma	H. ægyptium
			Rhipicephalus	R. bursa
			Dermacentor	D. reticulatus
				D. andersoni
			Margaropus	M. annulatus
			Amblyomma	A. hebræum
			Hæmaphysalis	H. leachi
Linguatulida			Linguatula	L. rhinaria
			Porocephalus	P. constrictus

The class Arachnoidea and the class Insecta belong to the phylum Arthropoda. This phylum contains a greater number of species than does any other phylum.

While the lobsters, crabs and water fleas, which belong to the class Crustacea, are important zoologically, they are of very slight importance medically. Besides the Crustacea we have the thousand-legged worms or Myriapoda.

The different classes of Arthropoda resemble the segmented worms but have as distinction the possession of jointed appendages which proceed from the somites in pairs. Some of the pairs of limbs are for locomotion; at times, certain ones may be specialized for food taking.

The somites or divisions of the body have a chitinous exoskeleton.

Respiration takes place through the medium of gills in the Crustacea and by tracheal tubes in the Myriapoda, Arachnoidea, and Insecta.

The Arachnoidea have no antennæ while the Myriapoda and Insecta have a single pair of antennæ, the former having numerous pairs of legs or jointed appendages while the latter have only three pairs of legs. The Arthropoda have segmented bodies, but they differ from the worms in having jointed appendages for the purpose of taking in food and moving from place to place. They also have an exoskeleton which is more or less unyielding from the deposit of chitin in the cuticle. This cuticle is not a true skin but only a secretion of the epidermis.

Within this external skeleton we have a dorsal digestive system and a ventral nervous system.

THE ARACHNOIDEA.

The Arachnoidea differ from the Insecta in having the head and thorax fused together. They also have four pairs of ambulatory appendages, while the insects only have three pairs. The Arachnoidea never have compound eyes—these when present being simple. Of the two orders of Arachnoidea of interest medically the Acarina is far more important than the Linguatulida.

Acarina.

Of the acarines we are chiefly interested in the mites and the ticks. The acarines do not show any separation of the abdomen from the cephalo-thorax. A hexapod larva develops from the egg; this is succeeded by an octopod nymph which differs from the adult in not having sexual organs.

In addition to the four pairs of legs in the fully developed acarine there are two other paired appendages, the cheliceræ. in front of the mouth, and the pedipalps on either side of the mouth.

Trombidiidæ.

These generally have a soft, more or less hairy integument and are often brightly colored. The two eyes are often pedunculated and the cheliceræ are lancet shaped and the palps project beyond the rostrum as claw-like appendages. A tip-like appendage on the apical segment of the palps is characteristic. A very common and annoying member of this family is the hexapod larva of the **Trombidium holosericeum.** It is usually designated Leptus autumnalis. Popularly it is termed "harvest mite," "red bug" or "jigger." They are found in the fields in the autumn

and attack both man and animals. The condition (itching and redness) produced is at times called autumnal erythema. There is a Trombidium in Mexico which has a predilection for the skin of the eyelids, prepuce, and navel. The Kedani mite, an orange-red larval mite about 250 by 125 microns is believed by the Japanese authorities to bring about infection with Japanese river fever or Tsutsugamushi, as the result of transmitting either a bacterium or protozoon by its bite. The disease somewhat resembles typhus, although an eschar at the site of the bite and lymphatic involvement is present.

Gamasidæ.

Of the Gamasidæ, which generally have a hard leathery body and styliform piercing cheliceræ, delicate five jointed palps and styliform hypostome, only the Dermanyssus gallinæ is of interest. This coleopterous mite infests chicken-houses and sucks the blood of the inmates. They will also attack man. Poultrymen may be troubled with a sort of eczema on the backs of the hands and forearms, similar to scabies, resulting from bites by these mites. They measure 350×650μ. They have no eyes.

Tyroglyphidæ.

Mites of this family live on cheese, flour, dried fruits, etc. They are small, without eyes, and have a smooth skin and a cone-like appearance of the mouth parts which are largely formed by the chelate cheliceræ. They are chiefly of importance because of their being occasionally found in urine, fæces, etc., and being striking objects, the question of pathogenicity arises. The T. longior has been associated with intestinal trouble (probably a coincidence, patient having eaten cheese containing these mites).

Glyciphagi are found in sugar and are the cause of what is known as "grocers' itch." Rhizoglyphus parasiticus is reported to be the cause of an itch-like affection of the feet of coolies on tea plantations. To distinguish: the dorsum of Glyciphagus is hairy or plumose; Tyroglyphus has both claws and suckers on tarsi, while Rhizoglyphus has only claws.

Sarcoptidæ.

These are small eyeless mites with a transversely striated cuticle. They live on the epidermis of man and various animals. The rostrum is chiefly made up of chelate cheliceræ with quite short three jointed, rather adherent palpi. It is the female that makes the tunnels in the skin between the fingers, on penis, flexor surface of forearm, etc. The male dies off after copulation. The female passes through four stages: 1. larva; 2. nymph; resembles adult, but has no sexual organs; 3. the pubescent female; 4. the egg-bearing female. A pair of itch mites may produce 1,500,000 descendants in three months. Transferrence of eggs, larvæ or pubescent females does not seem to transmit scabies. It is the egg-laden female only. The human itch mite, Sarcoptes scabiei, is an oval mite, the male is 250×150μ; the female is about 400×300μ. Besides the difference in size, the male may be distinguished from the female by the fact that the third and fourth pairs of legs in the female have

bristles, but in the male, the fourth pair has suckers. The tunnels made by the female have the egg-bearing female at the blind end; scattered all along are fæces, eggs, larvæ; the eggs being next the mother and the more mature young at the entrance to the gallery. A diagnosis can be made from the finding of either eggs or larvæ. The eggs are 140μ long and hatch out in four to five days. A female becomes mature in about two weeks.

In treating itch with sulphur preparations the adult females and immature itch mites are killed; the eggs, however, are not affected. Hence a second treatment about ten days after the first is necessary to kill the young mites, which have devel-

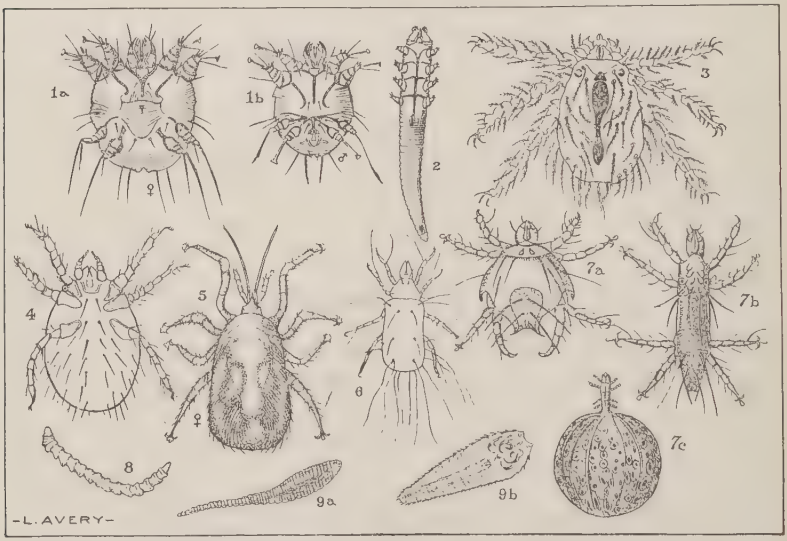


FIG. 78.—Arachnoidea exclusive of ticks. (1*a*) Sarcoptes scabiei, female; (1*b*) S. scabici, male; (2) Demodex folliculorum; (3) Trombidium akamushi, hexapod larva (Kedani mite); (4) Trombidium holosericeum larva (Leptus); (5) Dermanyssus gallinæ; (6) Tyroglyphus longior; (7*a*) Pudiculoides ventricosus, male; (7*b*) P. ventricosus, young female; (7*c*) P. ventricosus impregnated female; (8) Porocephalus armillatus; (9*a*) Linguatula serrata, female; (9*b*) L. serrata, larva.

oped subsequent to the first treatment. Different animals have different species of itch mites.

Demodicidæ (Hair Follicle Mites).

Demodex folliculorum.—This is a vermiform acarine about 400μ long; the eggs are about 75μ long; they chiefly live in the sebaceous glands of nose and forehead.

Tarsonemidæ.

This acarine family shows a complete sexual dimorphism. The Pediculoides ventricosus is oval and about 125×75μ for the male which has claws at the extremi-

ties of the anterior and posterior pairs of legs; the two other pairs have hooklets and a sucking disc. The female is about twice as long but of the same breadth as the male, and has claws only on the anterior legs.

The cheliceræ are needle like with inconspicuous palps and the front and rear pairs of legs are widely separated. The gravid female is like a ball and is about 1000μ in diameter.

They live on wheat and may be found in wheat straw, which, if handled, may be followed by a severe skin eruption with an irregular fever.

Ixodidæ.

This family of the Arachnoidea is one of great medical interest and of growing importance. It has recently been proposed to raise the ticks to a superfamily, Ixodoidea and to divide it into the families Argasidæ and Ixodidæ.

While only proven the intermediary hosts in the case of the organism of African tick fever and the as yet undiscovered cause of spotted fever of the Rocky Mountains, there is considerable speculation as to the possibility of blackwater fever being due to a Babesia (Piroplasma). Piroplasmata of animals seem to be invariably transmitted by ticks.

Very important diseases due to these small pear-shaped organisms within red cells are known for various animals, the best known being that of cattle in Texas and known as Texas fever. Other piroplasmata diseases are Rhodesian fever (cattle), heart water (sheep), and malignant jaundice of dogs. In these diseases there are pathological features which resemble blackwater fever of man.

It is of interest to note that it was with the transmission of Texas fever through an intermediate host (the tick) that Smith and Kilborne (1889–1893) established the zoological principle of transmission of disease through arthropod intermediary hosts. This led up to the work on malaria, yellow fever, etc.

Ticks differ from insects in having four pairs of legs, only two pairs of mouth parts, and no antennæ. They differ from other acarines in having a median probe-shaped puncturing organ, the hypostome, which is beset with numerous teeth projecting backward, and in possessing stigmal plates. The head, or capitulum, or rostrum, is the part which projects anteriorly from the body. This carries the piercing parts which are the single hypostome or dart and a pair of piercing chitinous structures, the cheliceræ which lie above the hypostome. As a sheath for these delicate biting parts we have a segmented pair of palpi or pedipalps. The mouth is a slit between the cheliceræ and hypostome.

Two depressed pitted areas on the dorsal surface of the capitulum in the adult female are known as porose areas. Very important structures are the stigmal plates. These are striking mosaic-like areas which are located just posterior to each hind leg in the Ixodinæ and between the third and fourth legs in the Argasinæ.

As the greatest confusion exists as to the classification of ticks, Dr. Charles W. Stiles has now in hand a system of classifying ticks according to the appearance of these plates as seen under the high power of a microscope. There is great variation in the outline and general picture of these stigmal plates in the different species. The stigmal orifice, the opening of the tracheal system, is in the center. The Ixodinæ have a scutum or shield-like chitinous structure on the dorsal surface. It covers almost the entire back of the tick in the male and only a small portion anteriorly in the female. The genital opening is toward the anterior part of the ventral surface. The anus, with anterior or posterior anal grooves, is near the posterior third of the venter. The legs have six segments, the coxa being flattened out on the surface of the body and the terminal tarsus ending with a pair of hooks and at times with a pulvillus. The nymph has stigmal plates but has no genital opening while the larva has neither genital apertures nor stigmal orifice.

Life History of Ticks.—This varies greatly according to the subfamily, genus, and species. The female Ornithodoros savignyi lays about 140 eggs. The larva does not leave the egg, but moults inside, and finally emerges as an eight-legged nymph. It lives in the dust in the cracks of the native huts and comes out at night to feed on the sleeping natives. As the possibilities for destruction are not so great as with many Ixodinæ the necessity for thousands of eggs is not imperative for the continuation of the species as with the Ixodinæ. With some of the Ixodinæ the females lay from 5000 to 20,000 eggs during several days or weeks and then die. The eggs are preferably deposited near grass. The egg stage lasts from two to six months, when the six-legged larva ("seed tick") emerges. It crawls up a blade of grass and gets on a passing animal. After feeding, or at times without taking nourishment, the larva drops to the ground, and changes to the pupal stage which has four pairs of legs. The pupa crawls up a blade of grass and gets on a passing animal (the second host). Feeding, it falls to the ground where it remains eight to ten weeks. It moults and develops into an adult tick. These males and females gain access to a third animal host—the males fecundate the females, after which the female gorges herself with blood; afterward dropping off the animal and laying eggs. With some ticks fewer hosts suffice.

Cleland has noted reports of serious symptoms, chiefly cardiac and visual, from the bite of ticks in Australia (Ixodes holocyclus). This is exceptional, however, as the symptoms following the bites of such ticks are only those of skin irritation.

Classification of Ixodidæ.

Subfamily Argasinæ.—Head concealed by body when viewed dorsally. No scutum. Stigmal plates between third and fourth legs.

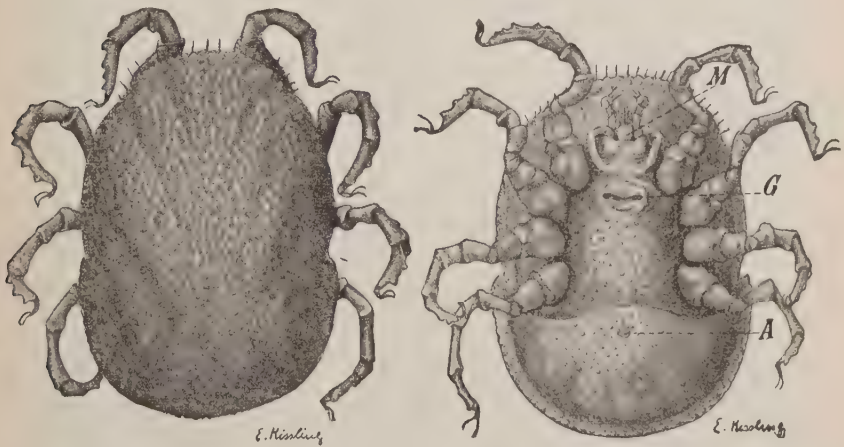
Adults have no suckers beneath claws. Slight sexual dimorphism. Anus near middle of venter. Skin rough.

Genus Argas.—Body narrow in front. Margins thin and acute. No eyes. The A. persicus (Miana bug) of Persia has been supposed to be concerned in the transmission of a serious disease. Rostrum some distance from anterior margin. It is also called the fowl tick and transmits fowl spirillosis.

Genus Ornithodoros.—Margins of body rounded. Skin has many irregular tubercles. Rostrum even with anterior margin so that ends of palpi slightly project. It is the intermediate host of Spirochæta duttoni. (South African tick fever.)

O. moubata is very common in Africa living in cracks in mud floors and bites severely the sleeping natives. The larva makes its first moult inside the egg so that it shows 4 pairs of legs when it emerges. Christy thinks it may transmit Filaria perstans.

O. savignyi has two pairs of eyes near base of mouth parts.



Eig. 79.—*Ornithodoros moubata.* (*Murray from Doflein.*)

Subfamily Ixodinæ.—Mouth parts project in front of body when viewed dorsally. Scutum present. Stigmal plates posterior to fourth pair of legs. Adults have suckers beneath claws. Skin finely striated.

Anus behind middle of venter.

Sexual dimorphism marked. Male has well-developed scutum; female has porose areas.

Section Ixodæ.—Transverse recurved preanal groove in female. Male has ventral surface covered with chitinous plates. No eyes. Genus Ixodes.

Ixodes has long rostrum with slender palpi—palpi narrow at base, leaving gap between them and hypostome.

Section Rhipicephalus.—No preanal, but postanal groove in female. Ventral

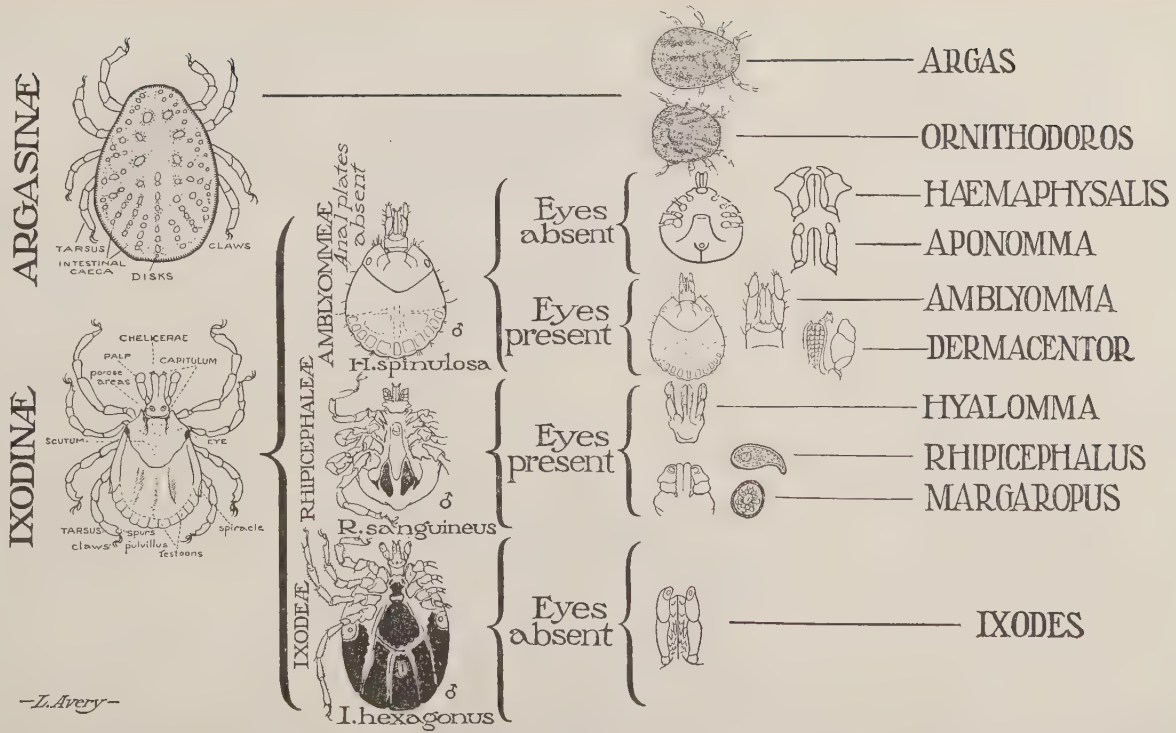


FIG. 80.—Diagrammatic key to ticks, modified from Lahille. Includes Ixodeæ (males clothed on all their ventral surface with anal plates in uneven numbers. Rostrum elongate. No eyes). Rhipicephaleæ. (Males having anal plates in pairs. Rostrum may be long or short. Eyes present.) Amblyommeæ. (Males without anal plates.)

surface of male without adanal plates in Dermacentor, Hæmaphysalis, Aponomma and Amblyomma, but with one or two pairs in Hyalomma, Rhipicephalus and Margaropus.

In the genera Hyalomma, Aponomma and Amblyomma the palpi are long and slender and of about uniform width of segments.

In Hyalomma the segments of palpi are of about equal length. In Aponomma and Amblyomma the second palpal segment is much longer than the others. Amblyomma differs from Aponomma in being very ornate and in having eyes.

In the genera Hæmaphysalis, Dermacentor, Rhipicephalus, and Margaropus the palpi are short.

Hæmaphysalis has very broad rostrum, triangular palpi, and no eyes. Dermacentor has a square rostrum with short thick palpi, the second and third joints being as broad as long. Dermacentor andersoni transmits spotted fever of the Rocky Mountains—not D. reticulatus.

Rhipicephalus has palpi without transverse ridges and comma-shaped stigmal plates. The stigmal plates of Margaropus are nearly circular and the palpi have acute transverse ridges externally. Margaropus annulatus transmits Texas fever of cattle. This tick is also called Boophilus bovis or B. annulatus. Some authors term it Rhipicephalus annulatus. Larvæ developing from eggs of female ticks which have fed on cattle infected with Texas fever transmit the disease which is due to a protozoon Babesia bigemina.

Linguatulida (Tongue Worms).

These are vermiform acarines more or less distinctly annulated. They have retractile hooks at either side of the elliptical mouth.

If the hooks are to be considered not as degenerated legs but antennæ and palpi, then there is no vestige of legs in the adult. The sexes are separate.

Linguatula rhinaria.—This has been observed in man both in larval and adult stages.

The male is white and about 3/4 inch long while the female is about 4 inches long, tadpole shape, yellowish in color, and has about ninety segments, lives in the nasal cavity and frontal sinus of dogs, rarely in horses and sheep, and very rarely in man.

The female lays embryo-containing eggs which, gaining freedom through the nasal mucus, are swallowed by various animals. A larva develops which bores its way through the gut and encysts in the liver or mesenteric glands. After several moultings, they work their way again to the intestines and so get out of the body of their host; or they may wander to lungs and trachea and either escape or take up their position in the nostrils to become adults and produce eggs. Consequently, one animal may act as intermediate and definitive host or these cycles may take place in distinct animal hosts.

The larval form (1/5 in.) is far more common in man than the adult. Symptoms are referred to liver in both larval and adult stage, and epistaxis and nasal symptoms for adult stage only.

Porocephalus constrictus.—The adult form P. moniliformis lives in the lungs of snakes and the eggs are probably ingested by drinking water. These eggs develop

into a curled-up, ringed larva, about 1/2 inch long with twenty-three rings, which is encysted especially in the liver or lungs. These escape and are swallowed by the snakes, their definitive hosts.

While in the liver or lungs of man the patient may have signs of bronchitis, hepatitis or peritonitis. Cases usually only discovered at postmortem. Parasites, however, might possibly be found in sputum or fæces.

CHAPTER XX.

THE INSECTS.

Classification of the Class Insecta.

Order.	Family.	Subfamily.	Genus.	Species.
Siphunculata	Pediculidæ		Pediculus	P. capitis
				P. vestimenti
			Phthirius	P. pubis
Rhynchota (Hemiptera)	Acanthiidæ		Acanthia	A. lectularia
	Reduviidæ		Conorhinus	C. megistus
				C. sanguisuga
Siphonaptera	Pulicidæ	Pulicinæ	Pulex	P. irritans
			Xenopsylla	X. cheopis
			Ceratophyllus	C. fasciatus
			Ctenocephalus	C. serraticeps
			Ctenopsylla	C. musculi
		Sarcopsyllinæ	Sarcopsylla	S. penetrans
Diptera	Simulidæ (buffalo gnats)		Simulium	S. reptans
	Psychodidæ (moth midges)		Phlebotomus	P. papatasii
	Chironomidæ (midges)		Ceratopogon	C. pulicaris
	Culicidæ	Culicinæ	Culex	C. fatigans
		Anophelinæ	Anopheles	A. maculipennis
	Tabanidæ (horseflies)		Tabanus	T. bovinus
			Hæmatopota	H. pluvialis
			Pangonia	P. beckeri
			Chrysops	C. dispar
			Glossina	G. palpalis
				G. morsitans
			Stomoxys	S. calcitrans
	Muscidæ		Musca	M. domestica
			Auchmeromyia	A. luteola
			Calliphora	C. vomitoria
			Lucilia	L. cæsar
			Chrysomia	C. macellaria (screw-worm)
	Sarcophagidæ		Sarcophaga	S. carnaria
			Ochromyia	O. anthropophaga
	Œstridæ		Dermatobia	D. cyaniventris
			Hypoderma	H. diana

INSECTA.

The class Insecta has one pair of antennæ, three pairs of mouth parts (the fused labium being considered as one pair), and three pairs of legs. They have three divisions of the body—head, thorax, and abdomen.

The head carries the antennæ and mouth parts; the thorax, which is divided into the pro-meso and meta thorax, carries upon the ventral surface of each thoracic segment a pair of legs and on the dorsal surfaces of the two posterior segments a pair of wings. The abdomen does not support appendages. The air is supplied by means of tracheæ—branching breathing tubes which have external openings or stigmata. The tracheæ are stiffened by spiral chitinous bands. The Malpighian tubules are excretory organs of the alimentary system and excrete nitrogenous waste material. Insects have two pairs of wings, the second pair of which is frequently rudimentary and shows simply as knob-like projections. These are termed halteres or balancers. In some insects both pairs of wings are rudimentary, as in Siphonaptera.

Where insects show metamorphosis we have voracious worm-like larvæ coming out of eggs; these larvæ are succeeded by a quiescent nonfeeding encased pupa which finally develops into an imago or fully developed insect. An insect which does not present this developmental cycle shows incomplete matamorphosis. Of the class Insecta only the Siphunculata Rhynchota, Siphonaptera, and Diptera are of special importance.

Siphunculata.

These are small flat wingless insects not showing metamorphosis.

The Pediculidæ.

In this family there are no wings and there is no metamorphosis. The acorn-shaped eggs (nits) are deposited on hairs of the host.

Pediculus capitis.—The female is about 1/12 of an inch long; the male smaller. They vary in color according to the color of the hair of the host. The eggs are deposited on the hairs of the head in number of 60 which hatch out in about six days. The thorax is as broad as the abdomen. The male louse is rounded off posteriorly and shows a dorsal aperture for a pointed penis, while the female is recognized by a deep notch at the apex of the last abdominal segment. There seems to be a marked preference exhibited by lice for their own peculiar racial host. It has recently been suggested that this might account for certain peculiarities in infection where different races were living together and under similar conditions as to food and environment, and yet only one race contracts the disease (beriberi). The head louse has been found to harbor leprosy bacilli when living on a leper.

Pediculus vestimenti.—This louse lives about the neck and trunk and deposits its eggs in the clothing. They number about 75 and hatch out in three or four days and become mature in about two weeks. Unlike the fleas there is no grub stage.

It is almost twice the size of the P. capitis and the abdominal segment is broader than the thorax. The abdomen is less markedly festooned than that of P. capitis; is less hairy and contains 8 segments as against 6 for P. capitis.

It has recently been shown to transmit typhus fever and more recently Nicolle has demonstrated it as a carrier of relapsing fever, the spirochætes being introduced by the material from the crushed louse being rubbed into the wound by the scratching of the victim (just as with the flea in plague) and not by the bite itself.

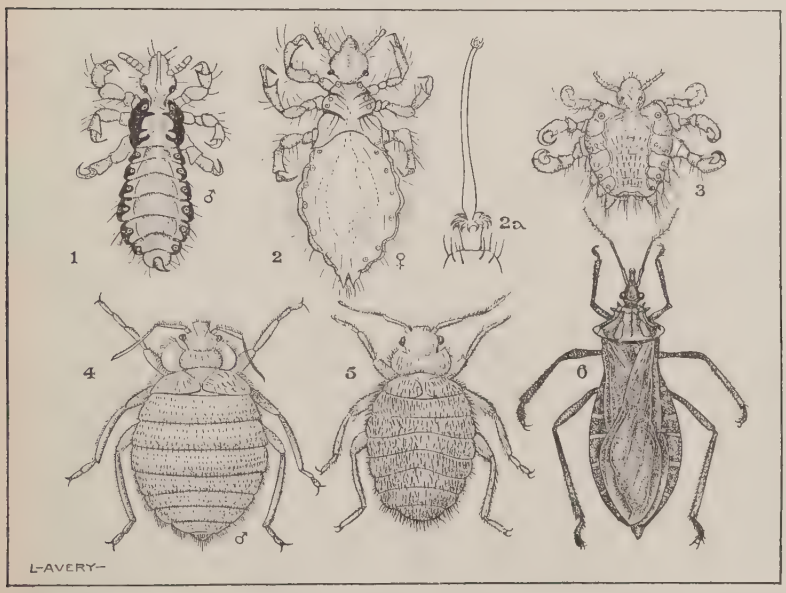


FIG. 81.—Siphunculata and Rhynchota. 1. Pediculus capitis. 2, Pediculus vestimenti. 2a. Protruded rostrum of Pediculus. 3. Phthirius pubis. 4. Acanthia lectularia. 5. A. rotundata. 6. Conorhinus megistus.

Phthirius pubis.—This louse is popularly known as the crab louse. The female is little more than 1/25 of an inch in length, and the male a trifle less. They are almost square. The second and third pair of legs are supplied with formidable hooks. They have a preference for the white race and live about the pubic region. The female lays about a dozen eggs, which hatch out in about a week.

RHYNCHOTA.

The Rhynchota are insects possessing a sucking beak in which the lower lip forms a long thin tube or rostrum which can be bent under the

head or thorax. Inside this tube are biting parts—mandibles and maxillæ. The metamorphosis in this order is not marked.

They have no palpi. The lower lip or labium or beak has it's edges curved to form the tube and it is only covered by the labrum at it's base. With the Diptera the labrum goes into the formation of the sucking tube. The mandibles and maxillæ are bristle-like structures serrated at the tip. The mandibles are grooved internally and form when apposed a tube for blood.

The Acanthiidæ.

These have a flattened body, a three-jointed rostrum, and four-jointed antennæ. Their wings are atrophied.

Acanthia lectularia (**Cimex lectularius**).—This is the cosmopolitan bedbug. It measures about 1/5 by 1/8 of an inch (5 by 3 mm.). It is of a brownish-red color. The most conspicuous feature of the bedbug is the long proboscis continuous with the dorsal integument of the head and tucked under the ventral surface. There are two prominent eyes and two four-jointed antennæ. There are eight abdominal segments. The bedbug lives in cracks and crevices, especially about beds. It is said thay can migrate from house to house. At any rate, they are frequently transferred with wash clothes. They have a penetrating odor when crushed. The female deposits about fifty eggs at a time in cracks and in ten days they hatch out into larvæ which pass insensibly into adults by a series of five moultings; this depositing of eggs occurs about four times a year.

The bedbug is very probably the intermediate host in kala azar and it has been incriminated in connection with typhus fever and relapsing fever.

In India the A. rotundata is the one encountered. It is of a dark mahogany color, has a smaller head, narrower abdomen, thick rounded prothoracic borders and is more densely covered with hairs than A. lectularia. The prothorax of A. lectularia is flattened at the side.

Reduviidæ.

These bugs have a long narrow head and a distinct neck. The antennæ are long and slender. The antennæ in the genus Conorhinus are inserted about midway between the eyes and point of the head.

Conorhinus sanguisuga.—This is known as the Texas or Mexican bedbug, and was formerly the foe of the common bedbug, but having gotten a taste for human blood through the Cimex or Acanthia, it now prefers man. It is extending toward the North. It has wings. The bites are much more severe than those of the common bedbug. It is of a dark brown color, nearly an inch in length, with a long, flat, narrow head and a short thick rostrum. They can run as well as fly. They bite at night.

Conorhinus megistus.—This is called "Barbeiro" in Brazil on account of its preference for biting the face. The Schizotrypanum cruzi undergoes a developmental cycle in this bug which transmits the disease.

Siphonaptera.

These are laterally flattened wingless insects and undergo a complete metamorphosis.

Pulicidæ.

This family is divided into two subfamilies—the Pulicinæ and the Sarcopsyllinæ. In the former the female remains practically unchanged after fecundation, in the latter the abdomen becomes enormously distended with eggs, and the female remains stationary after her impregnation in the burrow which she has made under the skin.

Pulicinæ.—Formerly, with the exception of infection with Dipylidium caninum, the fleas were only under suspicion as carriers of disease; ideas having been entertained as to their being possible transmitters of relapsing fever, typhus fever and kala azar. Trypanosoma lewisi is transmitted by fleas, either Pulex irritans or C. canis. The trypanosome undergoes development in the flea and the infecting material is in the fæces of the flea and transmission occurs by the licking on the part of the rat of fæces from an infected flea. The infection has no connection with the puncture wound of the flea as is the case with plague. As a result of the convincing experiments of the British Plague Commission, their rôle in the transmission of plague has been absolutely established. It is by the bite of the Xenopsylla cheopis that plague is chiefly transmitted from rat to rat, and in bubonic and septicæmic plague it is apparently the intermediary in human infection.

The average capacity of a flea's stomach is about 0.5 cmm. so that with a rat dying with septicæmic plague and with possibly 100 million bacilli to one c.c. of blood the flea would take in about 5000 bacilli. Furthermore these multiply in the alimentary canal so that the digested blood teems with bacilli when reaching the anus of the flea. The plague bacilli are passed out with the fæces and these being rubbed into the puncture of the flea bite bring about infection. The puncturing apparatus of the flea consists of a pointed epipharynx and two distally serrated mandibles. These chitinous biting parts are contained in the labium which divides distally into two labial palps. The maxillæ are conspicuous triangular structures and, projecting farthest anteriorly, are the conspicuous four-jointed maxillary palps, often mistaken for antennæ. By the apposition of the internally grooved mandibles to the epipharynx a tube is formed through which the blood is sucked up. The antennæ are inconspicuous and are in close apposition to the sides of the head, behind the eyes, and can only be well made out with a lens. Fleas have three pairs of legs, and the male can be distinguished from the female by its smaller size and the conspicuous coiled-up penis within the abdomen. The female has a conspicuous gourd-like spermatheca which varies in shape in different species. The body of

the flea is flattened laterally. They may or may not have eyes, and certain conspicuous structures called combs are of importance in classification. In the metamorphosis of the flea the eggs are hatched out in dust of crevices, etc., into bristled larvæ in about one week. The larva forms a cocoon and develops into a nymph which has three pairs of legs. The nymphs emerge from the cocoon as adult fleas in about three weeks after the larva forms it.

Key to the Fleas.

A. With combs.
  1. Eyes present.
    a. Combs along inferior border of head and on prothorax. *Ctenocephalus serraticeps.*
    b. Combs only on prothorax. *Ceratophyllus fasciatus.*
  2. Eyes absent.
    a. Collar of combs on prothorax and four short ones along inferior border of head. *Ctenopsylla musculi.*

B. Without combs.
  a. Ocular bristle arises near upper anterior margin of eye. A line between this and the oral bristle approximately vertical. Two bristles posterior to antennæ. Xenopsylla cheopis. *Læmopsylla cheopis.* Formerly *Pulex cheopis.*
  b. Ocular bristle arises near lower anterior margin of eye. A line between this and the oral bristle approximately horizontal. One bristle posterior to antennæ. *Pulex irritans.*

The common human flea of Europe is the Pulex irritans; that of the United States the Ctenocephalus serraticeps or dog flea. The flea that is implicated with plague is the Xenopsylla cheopis. It resembles P. irritans, but is more yellow than brown in color. It also has a greater number of bristles on the head. The ocular bristle runs above and in front of the eye; that of P. irritans below. It is principally the flea of Mus decumanus, the sewer rat; but the house rat, M. rattus, becomes infected from coming in contact with the sewer rat in the basement.

Ceratophyllus fasciatus is the common rat flea of Europe and the U. S. In the tropics X. cheopis is the common rat flea (98% in India). Ctenocephalus serraticeps, Ctenopsylla musculi and Pulex irritans have also been frequently found on both Mus norvegicus and M. rattus. To distinguish M. norvegicus from M. rattus we have in the former (1) ears which barely reach the eyes when laid forward and (2) tail rather shorter that length of head and body together (only 89% of length of head and body together). With M. rattus the tail is longer than the head and body together (25% longer) and the extended ear covers or reaches beyond the middle of the eye. M. rattus has a sharper nose, longer and more delicate tail and thinner ears than M. norvegicus (formerly M. decumanus).

M. alexandrinus is a variety of M. rattus. Rats and mice belong to the family

Muridæ and the common mouse is M. musculus. They belong to the order of Rodentia of the class Mammalia.

Sarcopsyllinæ.

Belonging to the subfamily Sarcopsyllinæ, the Sarcopsylla penetrans (Dermatophilus penetrans) is of great importance in tropical countries. It is known as the chigoe, nigua, or jigger. The male and virgin female are unimportant as they do not penetrate the skin but act as ordinary fleas. The female, which when unimpregnated is only about 1/24 of an inch long, when impregnated bores its way into the skin of man, especially about the toes, soles of the

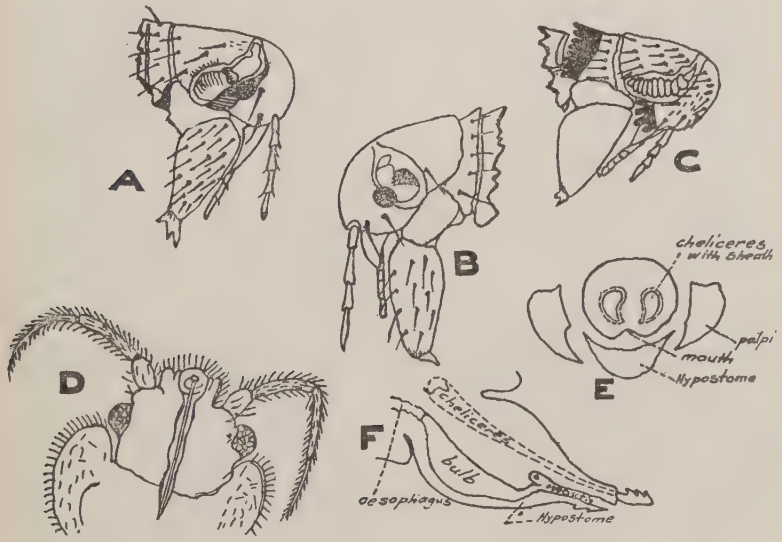


FIG. 82.—Fleas, bedbugs and ticks. A, Lœmopsylla cheopis; B, P. irritans; C, Ctenopsylla musculi; D, bedbug; E, cross section of rostrum of Ornithodorus; F. longitudinal section of Ornithodorus.

feet or finger-nails, and in the chosen site develops enormously, becoming as large as a small pea. This enlargement takes place in the second and third abdominal segments and is packed with eggs measuring about 400 microns long and numbering about 100. A small black spot in the center of a tense rather pale area is characteristic. The metamorphosis is similar to that of the flea. Sarcopsylla can be differentiated from the flea by the proportionately larger head to the body, and especially by the fact that the head is the shape of the head of a fish, distinctly pointed. With the fleas the lower border of the head comes out in a straight line to join the curve of the upper part. In the Sarcopsylla lower and upper border of head are both curved.

Diptera.

The insects of the order Diptera are of great importance medically in a variety of ways, either by the direct irritation of their bites, by their transmitting disease directly, as does the common house fly typhoid fever, or by acting as intermediate hosts for various parasites. They are characterized by mouth parts formed for puncturing, sucking, or licking. They present a complete metamorphosis, larva, pupa, and imago. As a rule, the Diptera have a distinct pair of wings, the second pair being rudimentary. With the Aphaniptera or Siphonaptera the

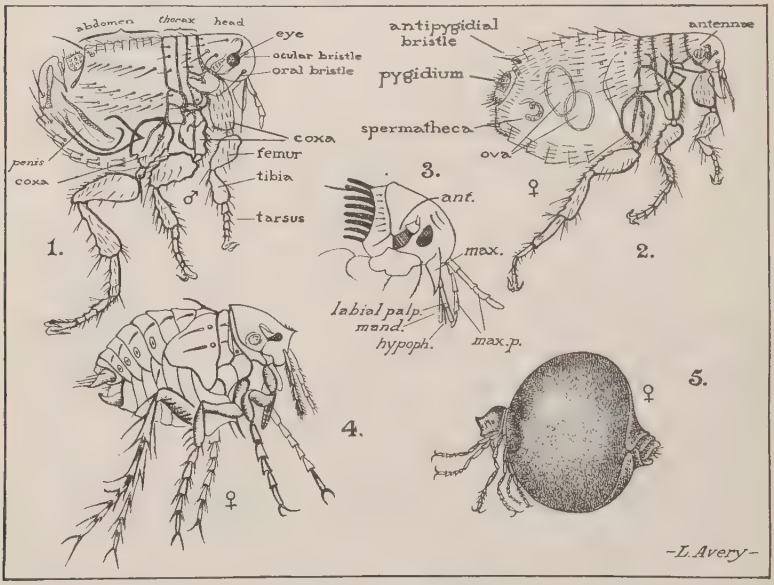


Fig. 83.—1 and 2, male and female Xenopsylla cheopis. 3, Head of Ceratophyllus. 4 and 5, male and egg distended female of Sarcopsylla penetrans.

wings are practically absent. Under the Aphaniptera, we have to consider the Pulicidæ or flea family.

The order Diptera is usually divided into the following suborders: 1. *Orthorrhapha:* Diptera with larvæ having a differentiated head. The imago breaks through the larval or pupal case by a T-shaped break and has no frontal lunule (an oval space just above the root of the antennæ). The Orthorrhapha are divided into: *a. Nemocera* (with long, many jointed antennæ) and *b. Brachycera* (with short antennæ) 2. *Cyclorrhapha:* larvæ without differentiated head. The imago escapes through an anterior opening and has a lunule and ptilinum (an inflatable projecting organ

just above the root of the antennæ). If the halteres are covered by a scale (squama) we have calyptrate Cyclorrhapha; if not, acalyptrate. These squamæ are large enough in the calyptrate species to even conceal the halteres when the fly is looked at from above. 3. *Pupipara:* the larvæ are extruded ready to begin the pupal state.

The males of flies where the two compound eyes come together above the antennæ are referred to as holoptic, if more or less widely separated as dichoptic. Ocelli are three single eyes usually, when present, situated in the triangular space between the compound eyes in the front (the space separating the compound eyes).

In studying the biting flies it is very important to recognize the anterior, small, or mid-cross vein. This short transverse rib or vein is the key to wing venation. Beneath it is the discal cell and it bounds the first posterior cell internally or basally. It is also of great value in differentiating Culicidæ. The character of the antennæ should also be noted carefully. The study of the bristles about head, thorax, and abdomen (chætotaxy) is more difficult. Anyone taking up the study of flies should carefully note the wings, etc., of Musca domestica. By putting a few house flies on moist horse manure in a gauze-covered bottle the entire metamorphosis may be observed.

Tabanidæ.

This is the family of horseflies, gadflies, breeze flies or green-headed flies. It is the most numerous family of the Diptera—there being more than 1000 species. The females are blood suckers; the males live on flowers and plant juices. The eyes are usually very brilliant in color, and in the male make up the greater part of the head.

They belong to the suborder Orthorrhapha and in the group of short antennæ flies (Brachycera). **Five posterior cells are always present.**

The antennæ consist of three segments. No arista. The epipharynx is tube like, the hypopharynx has a groove and both are awl shaped. The pair of maxillæ are serrated and the mandibles lancet like. They have rather coarse maxillary palps. The labellæ are prominent at the extremity of the fleshy labium. They are thick set flies and rarely show color. The body of the larva has eleven segments with a small but distinct head. The eggs are deposited in masses on the leaves or stems of plants about marshy places. The larva is carnivorous.

Tabanus autumnalis.—Is about 3/4 of an inch long; it is dark in color, and has four longitudinal bands on the thorax. The last joint of the antennæ has a crescentic notch. The wings do not overlap.

Hæmatopota pluvialis.—In the Hæmatopota there is no crescentic antennal notch, and the wings overlap. The abdomen is narrower than in Tabanus. The brimp, one of the Hæmatopota, bites man severely.

Pangonia beckeri.—The genus Pangonia is characterized by a very long, slender, and more or less horizontal proboscis.

Chrysops dispar.—Chrysops has three ocelli, in this respect differing from the genera Tabanus and Hæmatopota. The wings are widely separated and spotted. The antennæ of Chrysops are especially long and slender. Chrysops and Hæmatopota produce the greatest amount of pain from their bites. The Tabanidæ are not implicated as intermediate hosts in the transmission of disease. By their bites,

however, they may transmit disease directly, as with anthrax. Two species of Chrysops have been found to transmit Filaria loa.

Muscidæ.

The Muscidæ, Sarcophagidæ, and Œstridæ are calyptrate Cyclorrhapha.

The common housefly, **M. domestica,** is the best example of this family.

The arista is feathered both dorsally and ventrally with straight hairs. The fourth longitudinal vein bends down in a rather sharp angle as compared with Stomoxys, which gives the first posterior cell rather a fusiform appearance. The eyes are close together in the male, far apart in the female. The female lays about 125

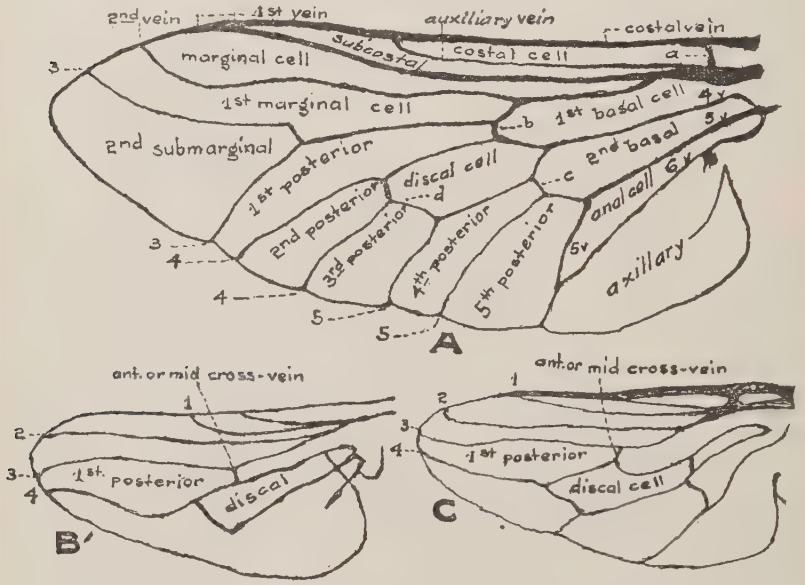


Fig. 84.—Wing venation of A, Tabanus; B, Stomoxys; C, Glossina.

eggs in a heap preferably in fermenting horse manure. The larva comes out in about thirty-six hours. Very characteristic are the stigmata decorating the blunt posterior ends. (See illustration.)

The larval stage lasts seven to ten days and then the barrel-shaped pupal stage is entered upon. This lasts about three days when the adult fly emerges. This fly is incapable of biting, the piercing organs being fused with the labium, but may transmit disease directly, carrying infectious material from the source, as in fæces, to the food about to be ingested. Their rôle in typhoid fever is one of immense importance. By reason of its hairy sticky legs, habits of frequent defecation and constant regurgitation the housefly is an important agent in the spread of cholera, dysentery, infantile diarrhœas and tropical ophthalmias as well as typhoid.

In the Muscidæ the antennæ hang down in front of the head in three segments and have an arista plumose to the tip. The first posterior cell is narrowed. There are no bristles on abdomen except at tip.

(I) Stomoxys, Hæmatobia and Glossina have a more or less elongated proboscis adapted for biting. Stomoxys has delicate palpi, shorter than the proboscis, and arista feathered only on the dorsal side with straight hairs. Hæmatobia has club-like palpi about as long as proboscis and arista with hairs dorsally and ventrally. Glossina has thick set but not clubbed palpi and an arista feathered on the dorsal side with branching hairs.

(II) Musca, Calliphora, Chrysomyia, Lucilia, and Cordylobia do not have a proboscis adapted for biting.

FIG. 85.—Common housefly (*Musca domestica*): Puparium at left; adult next, larva and enlarged parts at right. All enlarged. From circular 71 (by L. O. Howard), Bureau of Entomology, U. S. Department of Agriculture.

Stomoxys calcitrans.—These greatly resemble the common housefly in size and shape. They can be easily distinguished by the black, piercing proboscis extending beyond the head. There are longitudinal stripes on the thorax and spots on the abdomen. The proboscis on examination will be seen to be bent at an angle near its base. The palps are short and slender. The wings diverge widely.

The female lays about 60 banana-shaped eggs in horse manure. These hatch out in three days as larvæ which turn into pupæ in two or three weeks. After about ten days the fly emerges. The genus Stomoxys includes vicious biters. This is the fly which comes into houses before a rain, and which has given the common housefly the reputation of biting before a rain. Stomoxys may be implicated in transmitting surra (Trypanosoma evansi).

It has now assumed great importance as a transmitter of poliomyelitis and possibly of pellagra.

The horsefly (Hæmatobia irritans) rarely bites man. In these the palpi are much longer than in Stomoxys, being as long as proboscis. These palps are also thick and spatulate.

Glossina palpalis.—This is the tsetse fly that is responsible for the transmission of human trypanosomiasis (sleeping sickness).

The tsetse fly is a small brownish fly about 1/3 of an inch long. The proboscis extends vertically and has a bulb at its base. The arista is plumose only on the upper side and the individual hairs are themselves feathered. The wings are carried flat, closed over one another like the blades of a pair of scissors

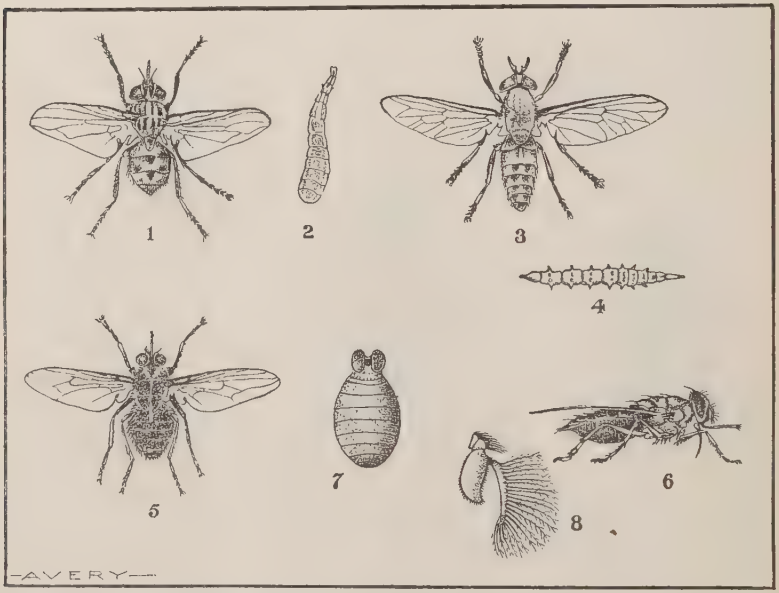


Fig. 86.—Insects in which the adult stage is important. (1) Stomoxys calcitrans; (2) S. calcitrans, larva; (3) Tabanus bovinus; (4) Tabanus larva; (5) Glossina palpalis; (6) G. palpalis, side view; (7) G. palpalis pupa; (8) Glossina palps and arista.

and project beyond the abdomen. The most characteristic feature of the tsetse fly is the way the fourth longitudinal vein bends up abruptly to meet the mid cross vein and then curves downward to run parallel with the third longitudinal vein. In Stomoxys, the wings separate; in Hæmatopota they just meet, and in Glossina they cross. Glossinæ bite chiefly in the daytime.

The tsetse fly does not lay eggs, but gives birth to a single full-grown larva almost as large as the mother which immediately bores its way into the soil and becomes a pupa.

The pupal stage is about a month and the larval stage in the mother about two weeks. G. palpalis bites in the day time. Both males and females bite. Glossina

morsitans transmits the cattle trypanosome disease, nagana and the human infection due to Trypanosoma rhodesiense.

Auchmeromyia luteola.—This is an African fly, the larva of which is known as the "Congo floor maggot," and is a blood sucker. The larva is of a dirty-white color and about 2/3 of an inch long. It crawls out at night and feeds on the sleeping natives. This is the only known instance of a blood-sucking larva.

Calliphora vomitoria and Lucilia cæsar.—These are flies with brilliant metallic-colored abdomens, commonly called blow flies in the case of Calliphora and blue-bottle flies for Lucilia. They deposit their eggs on tainted meat and in wounds.

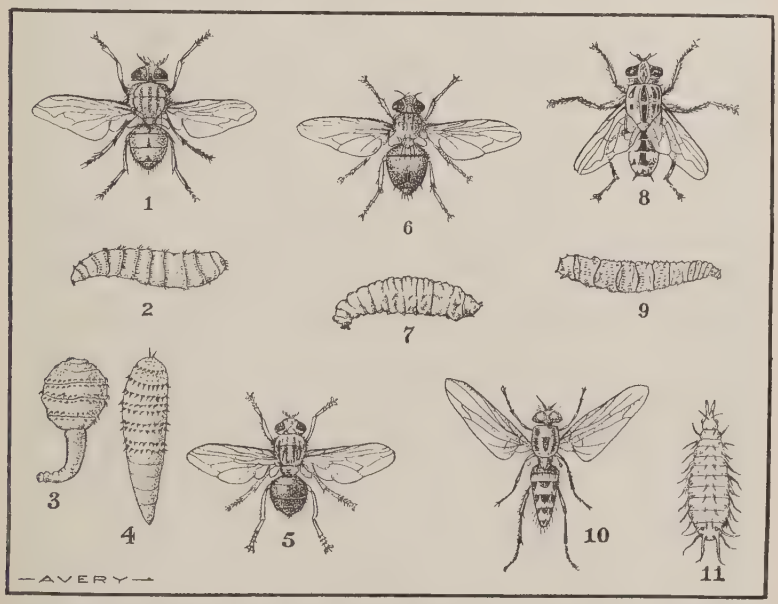


FIG. 87.—Insects in which the larval stage is important. (1) Chrysomyia macellaria; (2) C. larva; (3) Dermatobia cyaniventris larva, early stage (ver macaque); (4) D. cyaniventris larva, later stage (torcel or berne); (5) D. cyaniventris; (6) Auchmeromyia luteola; (7) A. luteola, larva; (8) Sarcophaga magnifica; (9) S. magnifica larva; (10) Anthomyia pluvialis; (11) A. pluvialis larva.

Many cases of obscure abdominal trouble are probably due to the larvæ of these flies. Intestinal myiasis is undoubtedly of greater importance than has been thought. The larvæ, with hook-like projections anteriorly and a ringed body, can easily be recognized in the fæces. They have been mistaken for flukes. They also have a tendency to be attracted by those with ozena and the larvæ may develop in the nostrils.

Chrysomyia macellaria.—This is known as the screw-worm when in the larval stage. The adult fly resembles the blue-bottle flies. It is distinguished from them, however, by the presence of black stripes on thorax. These flies are very common over nearly all North and South America. The thorax is striped. The eggs, which

number 250 or more, when deposited in the nostrils or in wounds, develop into the screw-worm larva, which may, by going up into the frontal sinus, cause death. These larvæ have twelve segments with rings of minute spines.

Ochromyia anthropophaga (**Cordylobia anthropophaga or Tumbu Fly**).—This is an African fly whose larvæ develop under the skin of man and animals. It is known as the Ver de Cayor. The larva resembles the Ver Macaque, is rather barrel shaped and beset with small spines. It bores its way into the skin and makes a lesion like a boil which has a central opening through which the larva breathes.

Sarcophagidæ.

These are known as "flesh flies." The most important characteristic is the fact that the arista is plumose up to the mid-point, beyond which it is bare. They are usually thick set and moderately large flies.

Sarcophaga carnaria.—This is a grayish fly with three stripes on thorax and black spots on each segment of the abdomen. It is viviparous. The larvæ gain access to nasal and other cavities and there develop. Cases of death have been reported. Naturally, the fly deposits its larvæ on decaying flesh. In times of war all of these flies become important by reason of "maggots" in the wound. These larvæ are the most common ones in intestinal myiases. The mouth hooklets are strongly curved and separate. Each abdominal segment has a girdle of spines. The anterior end is somewhat pointed. The hind stigmal plate is in a deep cavity.

Œstridæ.

The flies of this family are usually called botflies. The mouth parts are almost vestigial. They have a large head with a somewhat bloated-looking lower portion. They are often rather hairy. The larvæ which develop from the eggs are parasitic either in the alimentary canal or the subcutaneous tissues.

Dermatobia cyaniventris.—These are large, thick-set flies about 3/5 inch long, with prominent head and eyes, small antennæ, and a marked narrowing at the junction of thorax and abdomen. The thorax is grayish and the abdomen a metallic blue. The larvæ are deposited under the skin in various parts of the body. When the larvæ move they cause considerable pain. At first the larva is club-shaped, but later on it becomes oval. The former is called Ver Macaque, the latter Torcel.

Hypoderma diana.—The larval form of this fly has been reported three times for man. It forms tumors under the skin which it is thought may reach this location by proceeding in some way from the alimentary canal.

In Hypoderma the arista is bare while in Dermatobia the upper border is plumose.

CHAPTER XXI.

THE MOSQUITOES.

Mosquitoes (Culicidæ) are of the greatest importance medically, not only from their influence upon health in general by reason of interference with sleep and possibly from direct transmission of disease, but, more specifically, they are the only means by which it at present appears possible to bring about infection with such diseases as yellow fever, malaria, filariasis, and possibly dengue. In addition, many diseases of animals are transmitted by mosquitoes.

The Culicidæ differ from all other Diptera in having scales on their wings and generally on head, thorax, or abdomen.

To identify a mosquito, examine a wing and note the scales; also note the presence of two distinct fork cells and, in addition, that the costal vein passes completely around the border of the wing, making a sort of fringe with its scales. Mosquitoes undergo a complete metamorphosis, there developing from the egg a voracious, rapidly-growing larva; next, a nongrowing, nonfeeding stage—the pupa or nymph. There the head and thorax are combined in an oval body, from the back of which projects the siphon tubes; and tucked in ventrally is a small tail-like appendage.

The fully developed insect emerges from the pupa.

The Culicidæ belong to the suborder Nematocera. These have long articulated antennæ and include four families: Culicidæ Chironomidæ, Simulidæ, and Psychodidæ.

The principal mosquito-like, blood-sucking Diptera which are frequently mistaken for mosquitoes—none of which have scales on their wings—are the following:

1. **Chironomidæ** or Midges.—The blood-sucking species of Chironomidæ, which are found in most parts of the world, belong chiefly to the genus "Ceratopogon." These midges are of very small size, about 1/12 of an inch long, are able to get through netting and, usually being in swarms, they are exceedingly troublesome. The antennæ have thirteen joints and the wings are shorter than the abdomen and have only longitudinal veins. One of the midges, the "jejen" of Cuba, is a great scourge, its small size enabling it to enter eyes and nostrils. The larva of Chironomus is a red worm-like creature; the pupa has a tufted head.
2. **Simulidæ** or Buffalo Gnats.—These are small blood-thirsty insects only about 1/8 of an inch in length. The thorax is humped, the legs are short and the

proboscis short and inconspicuous. The antennæ have eleven joints but are rather short. One species, the S. damnosum, known by the natives of Uganda as "Mbwa," is greatly dreaded; its bites causing swellings and sores. Sambon has considered Simulium reptans as the transmitting agent of pellagra.

3. **Psychodidæ** or Moth Flies.—These are small, hairy, slender midges, with long legs and a short proboscis. The antennæ are long, hairy and consist of 12 to 16 joints. Palpi 4 jointed. They are only about 1/12 of an inch in length. The hairy wings have numerous longitudinal veins. Some, as

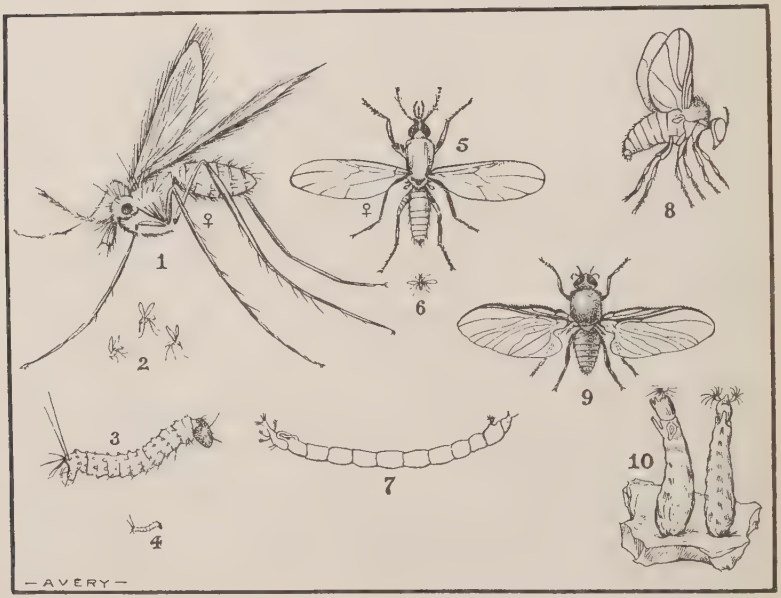


FIG. 88.—Mosquito-like insects belonging to families Chironomidæ, Simulidæ and Psychodidæ. (1)Phlebotomus papatasii; (2) P. papatasii (natural size); (3) P. papatasii (larva); (4) P. papatasii larva (natural size); (5) Ceratopogon pulicaris; (6) C. pulicaris (natural size); (7) Chironomus larva; (8) Attitude of a Simulium; (9) Simulium reptans; (10) Larvæ of Simulium.

Phlebotomus, have an enlongated proboscis and are vicious blood suckers. It has been suggested that they may be of importance in the transmission of tropical ulcer. A fever of about three days' duration found in Bosnia, characterized by leukopenia and similar to dengue and known as Phlebotomus or Pappataci fever, has been thought to be caused by the bite of infected P. papatasii.

Phlebotomus is common in the tropics and may transmit surra. The proboscis is much shorter than that of mosquitoes.

Mosquitoes have three main parts of the body—the head, the thorax, and the abdomen. On the head, the space behind the two

compound eyes is called the frons, in front, and the occiput posteriorly.

The nape is back of the occiput. The bulbous prolongation of the frons which projects over the attachment of the proboscis is the clypeus. The clypeus is hairy in the Culex; scaly in Stegomyia. The proboscis is straight in all mosquitoes of importance medically. It consists of a fleshy, scaled, gutter-shaped portion beneath, known as the labium, which terminates in two hinge-joint processes—the labella. At the end of the labium is a thin membrane (Dutton's membrane). It is through this that filarial embryos are supposed to pass on their way from the interior of the labium to enter the person bitten. The labium may be considered as the sheath of a knife, holding and protecting the slender, blade-like penetrating

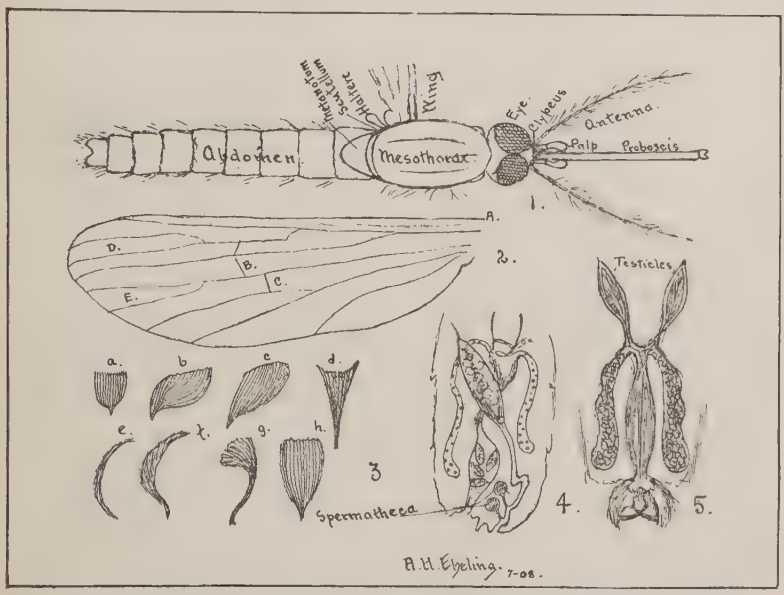


FIG. 89.—Anatomy of mosquito. 1, Dorsal view of mosquito; 2, wing of mosquito; A, costal vein; B, mid cross vein; C, posterior cross vein; D, first fork-cell; E, second fork-cell; 3, various types of scales; a, flat head scales; b and c, Mansonia wing scales; d, upright forked head scales; e, f, g and h, various shapes of thoracic scales.

organs. Lying in this groove we have, from above downward, the horseshoe-shaped labrum—epipharynx, the under surface of which is open. This when closed by the underlying hypopharynx forms a tube through which the blood is sucked up by the mosquito. In the hypopharynx, which somewhat resembles a hypodermic needle, is a channel, the veneno-salivary duct. It is down this channel that the malarial sporozoite passes. There are two pairs of mandibles and two pairs of maxillæ on either side of the hypopharynx—the mandibles above and the maxillæ below. The serrations of the maxillæ are coarser than those of the mandibles. The sensory organs, the palps, lie on either side of and slightly above the proboscis.

These are of the utmost importance in differentiating mosquitoes and must not be confused with the antennæ, which are attached above the palpi and at the sides of the clypeus. These antennæ are of importance in distinguishing the sex of the mosquito.

The thorax is largely made up of the mesothorax, at the posterior margin of which is a small, sharply-defined piece, the scutellum; this may be smooth or trilobed. Underneath and posterior to the scutellum is the metanotum; the metanotum is bare in Culicinæ, has hairs in Dendromyinæ and scales in Joblotinæ.

There is a pair of wings attached to the posterior part of the mesothorax and,

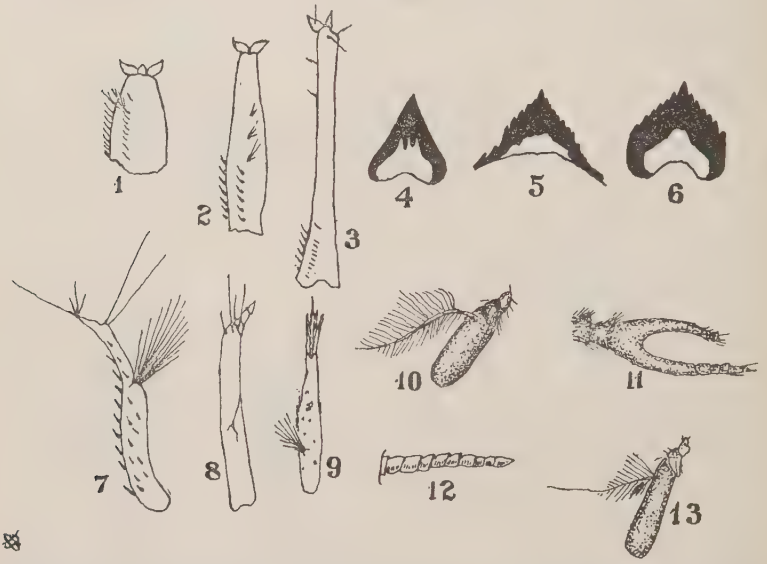


FIG. 90.—Distinguishing characteristics of mosquito larvæ and fly antennæ. Siphon tubes of 1, Stegomyia, 2, Culex, 3, Tæniorhynchus; mental plates of 4, Tæniorhynchus, 5, Stegomyia, 6, Culex; larval antennæ of 7, Culex, 8, Stegomyia, 9, Anopheles; antennæ of 10, Muscidæ, 11, Tabanidæ, 12, Simulidæ, 13, Sarcophagidæ.

more posteriorly still, a pair of rudimentary wings (halteres) attached to the metanotum. The three pairs of legs are attached to the thorax.

There are nine segments in the abdomen. The genitalia arise from the terminal segments as bilobed processes. In the male there is a pair of hook-like appendages or claspers, between which, and ventrally situated, are the harpes, also a pair of chitinous processes.

In considering the question of the possible danger which might arise from the introduction of a case of yellow fever, malaria, or filariasis, it would give the greatest information if mosquito ova were at hand so that we could by watching the development from egg to larva, pupa, and insect, have all the points from which to decide as to the genera developing in the given locality. It is generally a very easy matter

to dip out large numbers of larvæ from the pools and having noted the characteristics of the larvæ, to do the same when the pupæ develop; so that we have only to verify our identification when the insect emerges from the pupa.

The Ova.

The egg raft of Culex, containing about 250 ova, is quite perceptible on the surface of the water as a black, scooped-out mass, about 1/5 of an inch in length. The eggs are set vertically in the raft. The eggs of the Stegomyia are laid singly and have a pearl-necklace-like fringe around them.

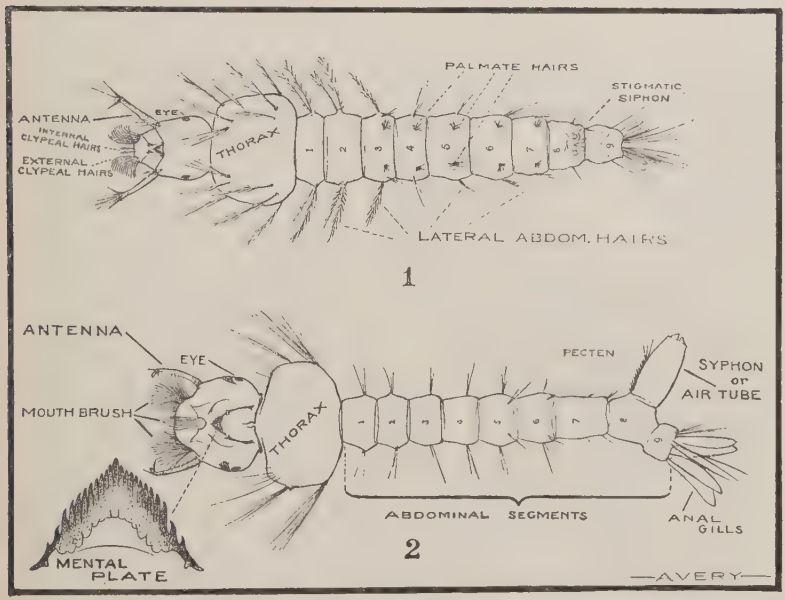


Fig. 91.—1. Asiphonate larva. Anopheles. 2. Siphonate larva. Stegomyia.

The Anophelinæ eggs are oval in shape with air-cell projections from either side. They are laid in triangle and ribbon patterns. The markings of these air cells vary and have been used for differentiation. The length of time of the egg stage varies according to temperature and other conditions—one to three days for Stegomyia and two to four days for Anophelinæ. The Anophelinæ are more difficult to raise than Culex or Stegomyia.

Larvæ.

There are two great classes of larvæ—the siphonate and the asiphonate. The latter are always Anophelinæ.

The Culicinæ larvæ have a projecting breathing tube at the posterior extremity which is called a respiratory siphon. This projects off at an angle from the axis

of the body, the true end of which terminates in four flap-like paddles. If you divide the length of the siphon by the breadth, you get what is known as the siphon index. In Culex the siphon is long and slender, in Stegomyia it is short and barrel-shaped. When at the surface the Culex larva has its siphon almost vertical and the body at an angle of about 45°.

The Stegomyia larva hangs more vertically. As a rule, the hairs proceeding from the sides of Culex larvæ are straight and the head relatively large. There are also no palmate hairs along the sides.

The Anophelinæ larvæ have a small head which is capable of being twisted around with lightning-like rapidity. They are darker in color and have no siphon;

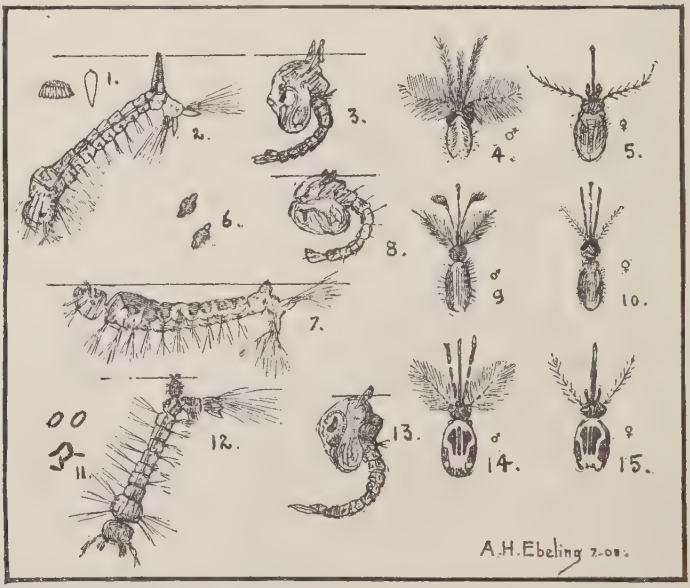


Fig. 92.—Metamorphosis of mosquitoes. 1, 2, 3, 4, and 5, Eggs, larva, pupa, and heads of male and female Culex; 6, 7, 8, 9, and 10, eggs, larva, pupa, and heads of male and female Anopheles; 11, 12, 13, 14, and 15, eggs, larva, pupa, and heads of male and female Stegomyia.

float parallel to the surface of the water; have long lateral branching hairs, and on the sides of each of the five or six middle abdominal segments they have a pair of palmate hairs. These palmate hairs are supposed to aid them in keeping their position on the surface of the water. The larvæ are usually called "wigglers." The duration of the larval stage is from 1 to 2 weeks, according to the temperature.

The Pupæ.

These have a bloated-looking cephalo-thorax and a shrimp-like tail—the latter the abdomen. Very important in examining them with a lens is to note the characteristics of the siphon tubes which project from the dorsal surface. These siphons

are long and slender in Culex and project from the posterior portion of the head end. In Anophelinæ they are broadly funnel-shaped and arise from the middle of the head end. The siphon of the Stegomyia is triangular.

The bulbous end of the Culex nymph is more vertical than the horizontally-placed cephalo-thorax of Anopheles. The duration of pupal life is short—only one to three days. At the end of this time the pupa comes to the surface and straightens out. The integument then splits dorsally and the perfect insect emerges. It dries its wings for a time on its raft-like pupal skin and then flies away.

From the above it will be seen that the stages in the metamorphosis of the mosquito take about two weeks: one to three days for egg stage; seven to ten days for larval stage, and two to three days for pupal stage.

Dissection of the Mosquito.

The easiest way to secure a mosquito for dissection is to use an ordinary plugged test-tube. Slipping the open end of the test-tube over the resting mosquito,

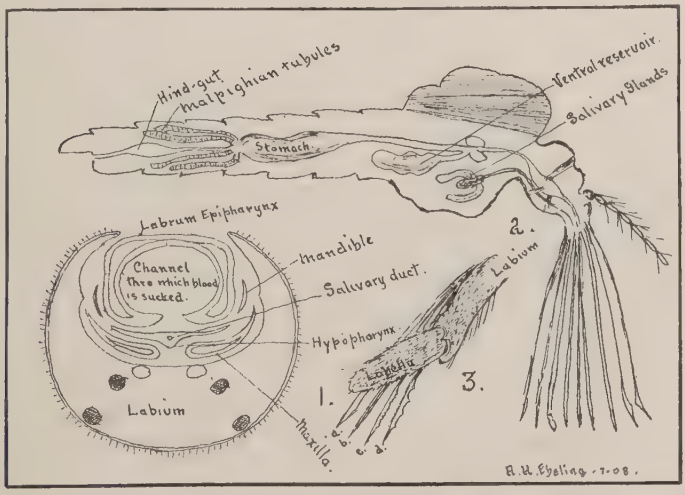


Fig. 93.—Anatomy of mosquito. 1, Cross section of proboscis of mosquito; 2, anatomy of mosquito, longitudinal section; 3, tip of proboscis of mosquito; a, labrum-epipharynx; b, hypopharynx; c, manidible; d, maxilla.

by a slight movement, the insect will fly toward the bottom. Then quickly insert the plug. If it is not desired to study the scales, the best way to kill the mosquito is by striking the tube sharply against the thigh. If it is also desired to study the scale characteristics it is better to put a drop or so of chloroform on the lower part of the cotton plug. The vapor falls to the bottom of the tube and kills the mosquito. Take the mosquito out, pull off legs and wings, and then place the body in a drop of salt solution on a slide. Bile has been recommended. Then hold the anterior end of the thorax by pressure of a needle. With a needle

in the other hand, gently crush the chitinous connection between the sixth and seventh segments of the abdomen. Then holding the thorax firm, steadily and gently pull the last segments in the opposite direction. If this is done properly, a delicate gelatinous white mass will slowly float out in the salt solution. One should be able to secure the alimentary canal as far up as the proventriculus, which is just anterior to the stomach, the part in which the malarial zygotes develop. Proceeding from before backward, we have the proventriculus, which is a sort of muscular ring at the opening of the stomach or mid-gut. Leading from the stomach we have the hind-gut, which ends in the rectum. Taking origin at the posterior end of the stomach and festooning the hind-gut are five longitudinal tubes—the Malpighian tubules. These are characterized by large granular-like cells with a prominent refractile nucleus. They are regarded as the renal structures. It is in these tubules that the embryo of the Filaria immitis of the dog develops. In the female mosquito, the parts withdrawn may seem to be largely made up of the white oval ovaries. These are connected with the spermathecæ, in which the spermatozoa are stored after fecundation by the male. In the male the testicles are quite distinct. Next to the examination of the stomach for zygotes, which appear as wart-like excrescences on its outer surface, the most important structures are the salivary glands, where the malarial sporozoites are found. The easiest way to dissect out the salivary glands is to press down firmly, but gently, on the anterior part of the thorax, and then with the shaft of a second needle, pressing on the head to gently draw the head away from the thorax, so that by this expression and traction movement you extract them with the head segment. They are very minute and are to be told by their exceedingly highly refractile appearance. To stain for sporozoites, pick up the head end, and with forceps draw the severed neck along a clean dry slide, trying at the same time to smear out the adherent salivary glands. After drying, stain with Wright's stain. The sporozoites are narrow falciform bodies about 12μ in length, with a central chromatin dot.

A matter about which there is dispute is as to whether the salivary glands communicate with the alimentary canal. Theobald states that there is no connection between them.

Differentiation of Culicinæ and Anophelinæ.

It is impossible even for an entomologist to differentiate mosquitoes without recourse to elaborate keys and tables. It is a comparatively easy matter, however, to decide as to whether the mosquito is a probable malaria transmitter or not.

While certain characteristics of the male are used to separate the Ædinæ from other subfamilies, yet it is only with the female that we concern ourselves in differentiating the Culicinæ from the Anophelinæ. Therefore, it is first necessary to distinguish the male from the female. If the antennæ have not been torn off, this can be decided by the highly adorned plumose antennæ of the male, those of the female being sparsely

decorated with short hairs. The palpi of the Anophelinæ tend to be clubbed, while those of the Culex are straight. If the antennæ have been broken off, look for the claspers at the end of the abdomen. Having determined that the insect is a female, we then proceed to place it either in the subfamily Culicinæ or Anophelinæ by a study of the relative length of the palpi to the proboscis. If the palpi are shorter than the proboscis, it belongs to the Culicinæ; if as long or longer, to the Anophelinæ. The palpi of the female Megarhininæ are also long, but the proboscis is curved.

Having settled on the subfamily, we separate the genera by considering such points as character and distribution of scales on back of head, wings, thorax, and abdomen; banding of proboscis, legs, abdomen, and thorax, shape of scales on wings, and location of cross-veins.

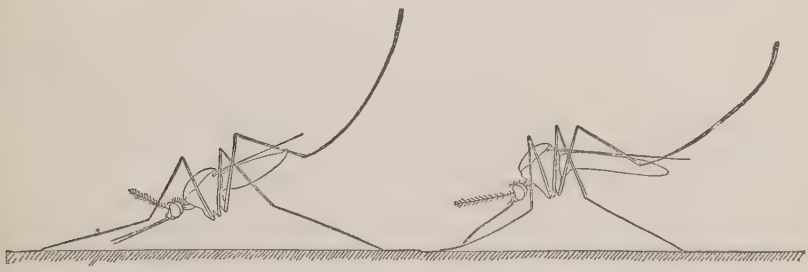


FIG. 94.—Anopheles. FIG. 95.—Culex.
Resting positions of anopheles and culex insects. (*Drawn by C. O. Waterhouse.*)

In the resting position Culex allows the abdomen to droop, so that it is parallel to the wall. The angle formed by the abdomen with head and proboscis gives a hunchback appearance.

Anopheles when resting on a wall goes out in a straight line at an angle of about 45°. It resembles a bradawl.

Classification.

There are four subfamilies of Culicidæ, differentiated according to the palpi:

1. Palpi as long or longer than proboscis in male.
   1. Palpi as long as proboscis in females; proboscis straight. *Anophelinæ.*
   2. Palpi as long or shorter than proboscis in females; proboscis curved. *Megarhininæ.*
   3. Palpi shorter than proboscis in females. *Culicinæ.*
2. Palpi shorter than proboscis in male and female. *Ædinæ.*

The important ones from a medical standpoint are the Anophelinæ and Culicinæ.

Anophelinæ.

1. Scales on head only; hairs on thorax and abdomen.	1. Scales on wings, large and lanceolate. *Anopheles.* Palpi only slightly scaled. 2. Wing scales small and narrow and lanceolate. *Myzomyia.* Only a few scales on palpi. 3. Large inflated wing scales. *Cycloleppteron.*
2. Scales on head and thorax (narrow curved scales). Abdomen with hairs.	1. Wing scales small and lanceolate. *Pyretophorus.*
3. Scales on head and thorax and abdomen. Palpi covered with thick scales.	1. Abdominal scales only on ventral surface. Thoracic scales like hairs. *Myzorhynchus.* Palpi rather heavily scaled.
	2. Abdominal scales narrow, curved or spindle-shaped. Abdominal scales as tufts and dorsal patches. *Nyssorhynchus.*
	3. Abdomen almost completely covered with scales and also having lateral tufts. *Cellia.*
	4. Abdomen completely scaled. *Aldrichia.*

Note.—Of the above genera only Cycloleppteron and Aldrichia are unproven malarial transmitters.

The Megarhininæ are of no importance medically.

The genus Megarhinus has the following characteristics:

1. Large mosquitoes with brilliant metallic coloring. (Elephant mosquitoes.)
2. Long, curved proboscis.
3. Caudal tufts of hairs on each side of abdomen.

The Ædinæ are not known to play any rôle in transmission of diseases. This subfamily is characterized by having the maxillary palpi much shorter in both males and females than the proboscis.

One genus Sabethes is very characteristic, owing to dense paddle-like scale structures on two or more legs.

Differentiation of Culicinæ Genera.

1. Posterior cross-vein nearer the base of the wing than the midcross-vein.
   1. Proboscis curved in female. *Psorophora.*
   2. Proboscis straight in female.
      A. Palps with three segments in the female.
         a. Third segment somewhat longer than the first two. *Culex.*
         b. The three segments equal in length. *Stegomyia.*
      B. Palps with four segments in the female.
         a. Palps shorter than the third of the proboscis. Spotted wings. *Theobaldia.*
         b. Palps longer than the third of the proboscis. Irregular scales on wings. *Mansonia.*
      C. Palps with five segments in the female. *Tæniorhynchus.*
2. Posterior cross-vein in line with midcross-vein. *Joblotina.*
3. Posterior cross-vein further from base of wing than midcross-vein. *Mucidus.*

Of the Culicinæ the genus Stegomyia is of importance on account of yellow fever. The totally efficient hosts for filariasis (filarial embryos found in the thorax and proboscis) are chiefly among the genus Culex. The genera Mansonia and Tæniorhynchus may also transmit filariasis. Some think the Anophelinæ genera "Cellia" and "Myzomyia" may transmit filariasis as well as malaria.

The genus Culex is implicated in dengue.

Stegomyia.—This is the most important culicine genus. These are mosquitoes with silver markings. The head, entirely covered with flat scales, has also some upright forked scales. Scutellum has dense flat scales. S. calopus is deep blackish-brown with two thoracic parallel lines with curved silver-white lines outside (lyre marking). Banding of thorax, abdomen, and legs.

S. calopus bites only at night after the first feeding. The first meal of blood however may be taken in the day time. To become infected it must take blood from a yellow-fever patient in the first two or three days of the disease. After sucking the blood of a yellow-fever patient the mosquitoes cannot transmit the disease by biting a nonimmune to yellow fever for a period of eleven days. After this time the mosquito remains infective for its life—in one instance 57 days.

S. scutellaris has a single silver stripe down the center of thorax. Mosquitoes of this genus are often called "Tiger mosquitoes." The larvæ have short, barrel-shaped siphons. They breed particularly in receptacles about the house.

S. pseudoscutellaris, which resembles S. scutellaris, but has white bands only, at the sides of the abdominal segments, is thought to transmit filariasis in Fiji.

Culex.—Male palpi long and acuminate. Head has narrow curved and up-

right forked scales. Laterally, flat scales. C. fatigans supposed to carry dengue as well as Filaria bancrofti. It also transmits Proteosoma of birds, the life history of which in this mosquito paved the way to the epochal discoveries in connection with malarial transmission by anophelines. This is a brown mosquito with pale yellow banding of each abdominal segment. The legs are brown except for the coxæ and femora.

Theobaldia.—These Culicinæ have spotted wings resembling Anophelinæ. These spots are due to aggregations of scales, not to dark scales. Male palps are clubbed (like Anopheles).

Mucidus.—This genus has a mouldy look from long twisted gray scales. The legs are densely scaled.

Mansonia.—This genus is characterized by broad flat asymmetrical wing scales. As the wing scales are brown and yellow the wings are mottled.

Grabhamia.—Wings have pepper-and-salt appearance with short fork cells.

Tæniorhynchus.—This genus is characterized by dense wing scales, which are broadly elongated with truncated apex.

Acartomyia.—Much like Grabhamia, but scales of head give ragged appearance. Male palpi clubbed.

A. zammittii was supposed to be concerned in Malta fever, but it is now known that transmission is by medium of milk of infected goats.

CHAPTER XXII.

POISONOUS SNAKES.

Snakes belong to the class Reptilia and the order Ophidia. They are divided into colubrine snakes (Colubridæ) and viperine snakes (Viperidæ).

Of the Colubridæ the Hydrophinæ or sea-snakes with rudder-like compressed tail and the Elapinæ with round tails are most important.

Many of our harmless snakes such as the garter-snake and blacksnake belong to the Colubridæ.

The cobras belong to the subfamily Elapinæ and are best known by a neck-like expansion or hood. The only poisonous colubrine snakes in the United States are the beadsnake (Elaps fulvius) often called the Florida coral snake, and the sonoran coral (Elaps euryxanthus).

The beadsnake is black with about seventeen broad crimson bands, which bands are bordered with yellow.

Although small, they are very venomous. The upper jaw has anteriorly grooved fangs, which appendages are not present in the nonpoisonous coral snakes, these latter having teeth in the upper jaw so that the wound shows four rows of punctures instead of two rows and one larger puncture on each side to mark the entrance of the fangs.

In Asia there are many important poisonous colubrine snakes; the cobra (Naja tripudians), the King cobra (Naja bungarus) and the Kraits (Bungarus fasciatus).

All of the Australian poisonous snakes are colubrines.

The Viperidæ which are characterized by a triangular head and tubular poison fangs are the most important poisonous snakes in America. The rattlesnake (Crotalus), the copperhead (Agkistrodon), and the water moccasin being widely distributed in the United States.

There are many harmless snakes which more or less resemble these "Pit Vipers" as the rattlers, moccasins, and copperheads are called. This term refers to a deep hole or pit found on the side of the head between the nostril and the eye. It is a blind sac.

Some divide the Viperidæ into the Crotalinæ, which possess the pit and the Viperinæ which do not have this structure.

The poison fangs are grooved or perforated and connected with the poison glands which resemble salivary glands and may be almost an inch in length in large snakes. The tongue is slender and forked and is a tactile organ.

The jaws are remarkable for their great extensibility, not only vertically, but laterally, by the ligamentous connections of the two halves of the mandible or lower jaw.

As the fangs are directed backward it is necessary for the snake when striking to open widely the jaws and bend back the neck. The fangs are then brought forward and erected by the spheno-pterygoid muscles. The snake bite is a combination of bite and blow. The functional fangs of colubrine snakes however are not mobile.

In addition to the possession of the pit, these vipers have a more or less triangular head and in particular a single row of large scales on the under surface posterior to the vent (anus), while the harmless snakes show an elongated oval head and two rows of large ventral scales posterior to the vent.

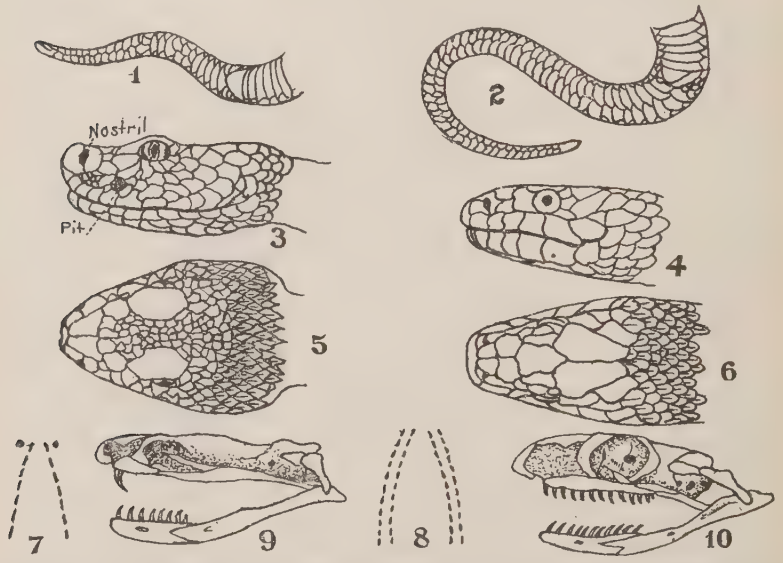


FIG. 96.—1, Single row of scales posterior to vent (poisonous snakes—water moccasin); 2, double row scales of harmless snake (Natrix); 3 and 5, side and dorsal view of head of pit viper; 4 and 6, side and dorsal view of head of harmless snake (Natrix); 7 and 9, bite puncture and skull of Elaps; 8 and 10, bite puncture and skull of harmless snake.

In examining the wound made by a snake the two punctures of the fangs indicate the bite of a poisonous snake. If these fang puncture points are far apart it shows that a large snake, and probably one capable of injecting a greater amount of venom has given the bite.

When a snake strikes the fangs move from the horizontal to the erect position, the mouth being widely open. When the fangs enter the jaws close and pressure is exerted on the poison glands so that the venom pours out.

The amount of venom varies with the size and condition of the snake, an adult cobra yielding about 1 c.c.

The cobra, after having bitten, remains attached for a short time while the daboia strikes with the greatest rapidity and immediately releases itself.

Cobra and krait bites (colubrine snakes) produce more or less similar symptoms such as paralysis of articulation with nausea and vomiting and later paralysis of the respiratory apparatus. There is only an insignificant reaction at the point of bite.

The venom is mainly neurotoxic, causing death by paralysis of cardiac and respiratory centers. Cobra venom is also very hæmolytic. This hæmolysin is activated by the normal complement of the serum of the animal poisoned, the hæmolysin as contained in the venom not being toxic when alone. Lecithin also has the property of activating the hemolytic amboceptor of venom.

In rattlesnake bites (viperine snakes) there is marked pain at the site of the wound with much swelling and hæmorrhagic infiltration. The swelling and petechial mottling spread up the limb from the point of entrance of the venom. Cold sweats, nausea, weak heart, and syncope are common.

Rattlesnake venom is active chiefly on account of it's hæmorahagin or rather endotheliolysin, which destroys the endothelial lining of blood-vessels.

Venoms may also contain proteolytic ferments which may account for the softening of muscles in snake bite cases. The toxic effect of the venom takes place without an appreciable incubation period, hence different from true toxins.

The most venomous snakes seem to be the sea-snakes (Enhydrina). This venom is almost entirely neurotoxic.

The tiger snake of Australia is almost equally venomous and the krait (B. cœruleus) next. The rattlesnake is about one-fifth as venomous as the krait.

Certain venoms greatly increase the coagulability of the blood so that intravascular thromboses may occur. It is chiefly with the venoms of Daboia and Bungarus that such thromboses are likely to occur and this accounts for the almost instantaneous death which at times results from bites of such snakes.

The nonspecific treatment of snake-bite poisoning is 1. by applying a tight ligature above the site of the bite. The ligature, which should preferably be a rubber band, is to be applied about a single bone extremity, not about one with two supporting bones. 2. The making of deep incisions about the fang punctures and thorough irrigation with a strong solution of potassium permanganate. Rogers has recommended that the punctures be enlarged with a lancet and the resulting wound packed with crystals of permanganate.

Recently Bannerman has shown that a dog bitten by a cobra cannot be saved by free incision and the rubbing in of permanganate crystals. It may however be saved by the immediate injection of 10 c.c. of a 5% solution of permanganate, but not if two minutes has elapsed. Bites from the daboia are fatal, however the permanganate be applied.

He therefore does not consider the permanganate treatment of any practical value. Rogers thinks that Bannerman's experiments with dogs do not give a true

idea of the value of permanganate because he has had success in experimenting with cats and because it has saved human lives. Chromic acid injections (1%) have also been recommended.

Internally alcohol does not seem to be of any value, in fact many of the deaths have been attributed to excessive ingestion of whiskey. Strychnine in large, almost poisonous doses, was highly recommended in Australia but the statistics seem to make the value of this remedy doubtful.

Antivenins.—The active agents of snake venoms may be either of the nature of hæmorrhagins, neurotoxins, or fibrin ferments. In colubrine snakes the neurotoxin vastly predominates while with the viperines it is the hæmorrhagin. Certain Australian snakes contain all three bodies in about equal proportion while with the rattlesnakes of America it is almost entirely the hæmorrhagin which causes the poisoning. The Elaps of Florida is a colubrine snake and its venom is neurotoxic in nature.

The cause of death in colubrine snake bites is chiefly from paralysis of the respiratory centers while with the Pit Vipers it is chiefly from hæmorrhages in the vital organs. Antitoxins have been prepared against both viperine and colubrine venoms and these are specific, a colubrine antivenin will not be of value against a viperine bite. Antivenins should be administered either intravenously or intramuscularly. The amounts recommended for injections to neutralize a fatal dose of snake poison vary from 100 to 300 c.c. of the antivenin serum. There is no accurate standardization.

PART IV.

CLINICAL BACTERIOLOGY AND ANIMAL PARASITOLOGY OF THE VARIOUS BODY FLUIDS AND ORGANS

CHAPTER XXIII.

DIAGNOSIS OF INFECTIONS OF THE OCULAR REGION.

It is advisable before taking material for cultures or smears to cleanse the nasal area of the eye-lids, and especially about the caruncles, with sterile salt solution. Then, by gently pressing on the lids, we may be able to get pure cultures of the organism causing the infection. Normally, we may find in the region of the caruncles various skin organisms, especially staphylococci, giving white colonies.

The xerosis bacillus and white staphylococci may be considered normal findings in the conjunctival sac. Streptococci and pneumococci have also been reported from apparently normal conjunctival secretions.

A small particle of sterile cotton, wound on a toothpick, with the aid of a sterile forceps, makes an excellent swab for obtaining material for smears; the same may first be drawn over an agar surface in a Petri dish in a series of parallel lines of inoculation before making the smears on slide or cover-glass.

When there is considerable discharge, a capillary pipette, with a rubber bulb, may be used to draw up sufficient material for cultures and smears. Be sure to round off the end of the pipette in the flame and not to use a very fine capillary tube.

In conjunctival cultures, plates of glycerine agar or agar plates smeared with blood are to be preferred, as the gonococcus and Koch-Weeks bacillus will only grow on blood or hydrocele agar. The diphtheria and xerosis bacilli grow well on glycerine agar.

In addition to the white staphylococcus, the streptococcus may be present when inflammation of the nasal duct exists.

The Streptococcus is at times responsible for a pseudo-membranous conjunctivitis. The Staphylococcus is as a rule the cause of phlyctenular conjunctivitis.

The pneumococcus is a fairly common cause of serpiginous corneal ulcerations. Active treatment is necessary.

It is now recognized as advisable to make an examination for the pneumococcus before performing operations on the eye as serious results may follow if the pneumococcus be present. It is the organism frequently found in dacryocystitis and, in the case of traumatism, may bring about panophthalmitis.

Corneal ulcerations are not apt to appear even with a pneumococcal conjunctivitis unless there be an injury of the epithelium.

The B. xerosis is possibly a harmless organism and must not be accepted as explaining an infection unless other factors have been eliminated. The true diphtheria bacillus, which the xerosis so much resembles, may cause a pseudomembranous inflammation.

The B. pyocyaneus may cause severe purulent keratitis as well as conjunctivitis. The pyocyaneus toxin appears to be a factor.

The gonococcus and the Koch-Weeks bacillus are usually responsible for the very acute cases of conjunctivitis. Both these organisms are characteristically intracellular and are Gram negative.

Conjunctivitis in the course of epidemic cerebrospinal meningitis has been found to be due to the meningococcus.

The diplobacillus of Morax and Axenfeld is more common in chronic, rather dry affections of the conjunctiva, chiefly involving the internal angle and showing a morning accumulation of the secretion. The bacilli are found in twos, more rarely in short chains. They are generally free but may be found in phagocytic cells. They resemble Friedländer's bacillus morphologically but do not have capsules.

In cases of ozena with involvement of the nasal ducts Friedländer's bacillus may be found.

Even in cases without ozena, capsulated, Gram negative bacilli of the Friedländer group have been frequently reported in conjunctival inflammation and in dacryocystitis as well.

The nodules of the eye-brows give the most convenient area to take material from in the diagnosis of leprosy, either the fluid expressed after scraping or a piece of tissue cut into sections. Conjunctival ulceration in leprosy may show abundant bacilli as is also true of corneal ulceration.

Ordinarily it is impossible to find tubercle bacilli in tuberculous conjunctival discharges.

The discharge from a tuberculous dacryocystitis may show them satisfactorily. Animal inoculation is preferable in the diagnosis of ocular T. B. The pneumococcus is, however, the most important organism in dacryocystitis—rarely the B. coli.

In a gonorrhœal ophthalmia the secretion is much more abundant and there is an absence of contaminating organisms, the reverse of infection with the confusing M. catarrhalis. As a matter of fact, large numbers of M. catarrhalis may be present in the conjunctival secretion with only slight irritation being observable.

In keratomycosis the cause has been ascribed to Aspergillus fumigatus.

Certain fungi of the genus Microsporum have been thought to be the cause of trachoma, as have also certain bacillary forms. One should be very conservative about reporting fungi in smears or cultures of external surfaces.

The larval stage of Tænia solium (Cysticercus cellulosæ) has a predilection for eye as well as brain. It is usually situated beneath the retina.

The question as to the nature of the so-called ophthalmic flukes is taken up under trematodes. Echinococcus cysts have been reported in the orbit.

The adult Filaria loa tends at times to appear under the conjunctiva or in the subcutaneous tissue of the eye-lids.

Fly larvæ have been reported from the conjunctival sacs in the helpless sick.

Demodex may cause an obstinate blepharitis.

Prowazek has thought that certain fine dots within the cytoplasm of epithelial cells, which stain best by Giemsa's method and which he considered protozoal in nature, were the cause of trachoma.

CHAPTER XXIV.

DIAGNOSIS OF INFECTIONS OF THE NASAL AND AURAL CAVITIES.

In taking material from the nasal cavities, for bacteriological examination, it is well to wash about the alæ with sterile water and then have the patient blow his nose on a piece of sterile gauze and take the material for culture or smear from this. If the material is purulent and located at some ulcerating spot, it is best to use a speculum, and either touch the spot with a sterile swab or use a capillary bulb pipette with a slight bend at the end.

Normally, we find only white staphylococcus colonies and colonies of short-chain streptococci. The M. tetragenus, B. xerosis, and Hoffman's bacillus are also occasionally found.

In some cases of ozena we may find an organism of the Friedländer type in pure culture.

Biscuit-shaped diplococci, both Gram negative and positive, are to be found either normally or in cases of coryza. M. catarrhalis has probably been frequently reported as the meningococcus. Still, the meningococcus has been found in the nasal secretions of patients with cerebrospinal meningitis. B. influenzæ and the pneumococcus have also been frequently found in cultures from the nasal secretions.

Diphtheria involving the nasal cavity must always be kept in mind, and in quarantine investigations the examination of the nasal secretions culturally should be a part of the routine.

The tubercle bacillus may be found in nasal ulcerations; it is, however, only present in exceedingly small numbers. On the other hand, one of the best diagnostic procedures in leprosy is to examine smears from nasal mucous membrane for the B. lepræ. In such ulcerations the bacilli are found in the greatest profusion. Rarely glanders may cause ulcerations.

B. proteus is frequently responsible for the production of foul odors in nasal discharges but does not seem to produce inflammatory conditions of the nasal mucosa. It simply decomposes the discharges. Various fungi have been reported from the nose, but in such a region the strictest conservatism in reporting should be observed.

Recently sporozoa have been reported in a case of nasal polyp. (Rhinosporidium.)

So many degenerative changes in epithelial cells resemble protozoal forms that such findings require ample confirmation.

The larval form of Linguatula rhinaria is a rare parasite of the nasal cavities; it is not infrequent, however, in the nostrils of dogs.

Various fly larvæ are far more common, and the "screw-worm," the larva of the Chrysomyia macellaria, is common in certain parts of tropical America, and may by its burrowing effects cause fatal results.

The larvæ of Sarcophaga have in particular been found in the nasal cavities of children. Myriapods, while of very little importance elsewhere, have been reported more than thirty times from the nasal fossæ.

In a study of the bacteriology of otitis media, in 277 cases, Libman and Celler found streptococci present alone in 81%, streptococcus mucosus in 10% and the pneumococcus in 8%; staphyococci, B. pyocyaneus and B. proteus have also been found. Mixed infections are common.

Streptococci are the organisms which most often cause sinus thrombosis and brain abscess. The influenza bacillus has been reported as a cause of acute otitis media.

Nonvirulent diphtheroid bacilli are not infrequently obtained in cultures from ear discharges.

Other organisms which have been isolated from middle ear or mastoid discharges are B. coli, M. catarrhalis, M. tetragenus and Friedländer's bacillus.

B. typhosus may be found in middle-ear discharges of persons who have had an attack of typhoid fever.

The middle ear is normally free of bacteria, but in affections of the throat, as with streptococci, pneumococci, and diphtheria bacilli, these organisms may infect it by way of the Eustachian tube.

The moulds are of greater importance in affections of the external auditory canal than the bacteria. The cerumen seems to make a good culture medium so that various species of Aspergillus, Mucor, etc., develop and close the canal. These infections are often introduced by the patient's finger. Various mites and fly larvæ have been reported from the ear.

CHAPTER XXV.

EXAMINATION OF BUCCAL AND PHARYNGEAL MATERIAL.

In a preparation made from material taken by a sterile swab from the region of the normal buccal and pharyngeal cavities and stained by Gram's method we are struck by the variety of organisms present.

Gram positive and Gram negative staphylococci are present, as are also streptococci, pneumococci, leptothrix forms, and very probably yeasts and sarcinæ types with many Gram negative bacilli. If pseudo-diphtheria organisms are present, we have these showing a Gram positive reaction. If this material is smeared on agar plates and cultured at 37° C., we are struck by the fact that the colonies on the plates may be exclusively staphylococcal and streptococcal.

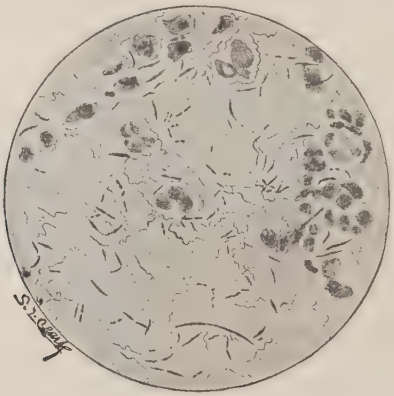


Fig. 97.—Vincent's angina. Spirochæta vincenti. (*Coplin.*)

It is very difficult, if not impossible, to distinguish a pneumococcus colony from a streptococcus one on a plate culture. The presence or absence, however, of the pneumococcus is distinctly shown in the Gram-stained smear, either by its lance-shaped morphology or the presence of a capsule. It has been my experience that smears from about 15% of normal individuals show capsulated pneumococci.

In diphtheria examinations we rely chiefly on the cultural findings on Löffler's serum. Where the process is streptococcal or due to the organisms associated with Vincent's angina, the immediate examination of a smear from the suspected spot or area gives greater diagnostic information. The streptococcus being so abundant in cultures from normal throats, it is difficult to determine its significance in a cul-

ture; abundance of streptococci in a smear from an ulceration or bit of membrane, however, is of etiological import.

By staining with Neisser's method it is possible to make an immediate diagnosis of diphtheria from a smear from a piece of membrane in about 25 % of cases. It is well, however, to always culture such material. The toluidin blue stain of Ponder is the best stain for diphtheria.

Material from the throat is ordinarily best obtained with a sterile copper-wire cotton-pledget swab. The platinum loop usually bends too easily. A sterile forceps may be more convenient for obtaining particles of membrane. It is believed that ulcerative conditions of the throat, associated with the presence of the large fusiform bacillus and delicate spirillum, which make the picture of Vincent's angina, are more common than is usually so considered.

In Giemsa stained smears from the dirty membrane covering the ulcerated area of Vincent's angina there are usually two types of the fusiform bacillus to be seen; one rather slender, pale blue with maroon dots at either end, the other rather thicker and of a uniform maroon staining. The spirilla are from 10 to 18 microns long and the fusiform bacilli from 5 to 7 microns.

As a rule, only cultures on serum are made and very rarely direct smears. If a smear were always made and stained by Gram's method (with a contrast stain of dilute carbol fuchsin) at the same time the culture was made, it is probable that much information of value would be obtained.

The B. fusiformis is an anaerobe which gives a fetid odor but culturally has no distinct characteristics. The spirillum has not been cultivated. It has been thought that the bacillus and spirillum are different stages of the same organism.

At times aggregations of the fusiform bacillus give the appearance of branching so characteristic of diphtheria organisms. Being Gram negative, however, the differentiation is easily made—the B. diphtheriæ being Gram positive. Again the attenuated ends of the fusiform bacillus are diagnostic.

Direct smears are the procedure of choice in streptococcal and pneumococcal anginas as well as in Vincent's angina.

Unless very familiar with the morphology of Treponema pallidum and using Giemsa's staining procedure, we should be very conservative in reporting such an organism from suspected syphilitic ulcerations of the throat.

The thrush fungus (Endomyces albicans) may be easily demonstrated in a Gram-stained specimen as violet mycelial structures.

Yeasts due to food particles are not infrequently observed in smears and cultures from the mouth.

Actinomycosis may develop about a carious tooth and the finding of the ray fungus in the granules from the pus may give the diagnosis.

Amœbæ and flagellates have been reported from the mouth. Also in the remarkable disease "halzoun," flukes have been found to be the cause of the asphyxia.

In the tropics, round worms may be vomited up and, lodging in the pharynx, may have to be extracted.

During the campaign of Napoleon in Egypt many cases of leech involvement of the nasal and buccal cavities were noted. The parasite was the Limnatis nilotica which gained access to the upper pharynx through drinking water from springs and pools. Many such cases continue to be reported from the Mediterranean basin.

CHAPTER XXVI.

EXAMINATION OF SPUTUM.

Frequently the material submitted for examination as sputum is simply buccal or pharyngeal secretion, or more probably secretion from the nasopharynx, which has been secured by hawking. It should always be insisted upon that the sputum be raised by a true pulmonary coughing act, and not expelled with the hacking cough so frequently associated with an elongated uvula. When there is an effort to deceive, some information may be obtained from the watery, stringy, mucoid character of the buccopharyngeal material and also from the presence of mosaic-like groups of flat epithelial cells (often packed with bacteria). The pulmonary secretion is either frothy mucus or mucopurulent material, and if the cells are alveolar they greatly resemble the plasma cells. At times these cells may contain blood pigment granules (heart-disease cells).

In the microscopic examination a small, cheesy particle, the size of a pin head, should be selected. This should be flattened out in a thin layer between the slide and cover-glass and should be examined for elastic tissue, heart-disease cells, eggs of animal parasites, amœbæ, and fungi. Echinococcus hooklets, Curschman spirals besprinkled with Charcot-Leyden crystals, and hæmatoidin and fatty acid crystals may also be observed.

Curschman spirals indicate bronchial as against cardiac or uremic asthma. Charcot-Leyden crystals have no special significance, except in certain tropical diseases when these crystals often are present in paragonomiasis sputum and in the pus of amœbic liver abscesses discharging by way of the lungs.

It may facilitate the examination of the sputum for elastic tissue and actinomycosis and other fungi to add 10% sodium hydrate to the preparation.

To make smears for staining, the sputum should be poured on a flat surface, preferably a Petri dish, and a bit of mucopurulent material selected with forceps. A dark back-ground facilitates picking out the particle. A toothpick is well adapted to smearing out such material on a slide. After using the toothpick it can be burned. When dry, the smear is best fixed by pouring a few drops of alcohol on the slide, allowing this to run over the surface, and then, after dashing off the excess of alcohol, to ignite that remaining on the film in the flame and allow to burn out.

A mark with a grease pencil, about 1/2 inch from the end, gives a convenient surface to hold with the forceps and also prevents the stain subsequently used from running over the entire surface. A piece of glass tubing about 12 inches long bent

into a narrow V shape makes a very satisfactory rest for the slide in staining and is convenient for the steaming of staining solution over the flame.

Sputum should as a routine measure be stained by the Ziehl-Neelson method and by Gram's method.

In examining for tubercle bacilli it may be necessary to employ some method for concentrating the bacterial content of the sputum prior to making the smear. A very satisfactory method is that of Mühlhäuser-Czaplewski. Shake up the sputum with four to eight times its volume of 1/4% solution of sodium hydrate in a stoppered bottle. When the mixture has become a smooth, mucilaginous-looking fluid, add a few drops of phenolphthalein solution and bring the pink mixture to a boil.

Then add drop by drop a 2% solution of acetic acid, stirring constantly, until the pink color is just discharged. If the least excess of acid is added over that just sufficient to cause the pink color to disappear, mucin will be precipitated. Now pour this mixture into a centrifuge tube and smear the sediment on a slide and stain for tubercle bacilli.

Tubercle bacilli usually occur nested in clumps of sputum. Therefore, when few in number it is only by chance that they may be found. Concentration methods aim to dissolve these clumps of sputum and collect, free from mucus, whatever bacilli may be present. There are many concentration methods for sputum. One of these has been given above. Uhlenhuth's method has some advantages over others in the solvent used: 1. It breaks up the sputum very rapidly; 2. it immediately dissolves all organisms except acid-fast ones; 3. applied in not too concentrated form and for not too long a time, tubercle bacilli are not killed, so that by washing the sediment carefully by several dilutions and centrifugings we have in the sediment viable tubercle bacilli which we may attempt to cultivate upon Dorset's or other suitable media with the reasonable hope that contaminations will not choke them out or prematurely kill the inoculated guinea-pig; 4. it has less effect upon the staining properties of tubercle bacilli than any other material used in concentration methods.

To make this solvent (antiformin) take double the quantity of chlorinated lime and sodium carbonate required by the U. S. Pharmacopœia and prepare according to U. S. P. directions. To the finished liquor sodæ chlorinatæ (Labarraque's solution) add 7 1/2% of sodium hydrate.

The Liquor sodæ chlorinatæ of the Br. P. is slightly stronger and some English authorities recommend a mixture of equal parts of this Labarraque's solution and 15% sodium hydrate solution. As a rule one part of antiformin to five parts of sputum is sufficient. Very tenacious sputum may require one part to four parts of sputum. If more antiformin is used the specific gravity is too much increased and the bacilli are damaged. The fluidification is hastened at incubator temperature.

To five parts of sputum add one part of antiformin, shake well and place in

incubator for one hour. To 10 c.c. of the homogeneous mixture add 1.5 c.c. of a solution made up of one part chloroform and nine parts alcohol. Shake violently and centrifuge for 15 minutes. Mix the sediment with egg albumin, smear out and stain.

When it is desired to culture the tubercle bacilli mix 20 c.c. of sputum with 65 c.c. sterile water and add 15 c.c. antiformin. Stir the mixture with a glass rod. After 30 minutes to two hours we should have a homogeneous mixture. Centrifuge for 15 minutes or longer, wash the sediment twice with sterile salt solution and smear out the well-washed sediment over serum or glycerine egg slants. The tubes should be covered with black paper and the plugs paraffined. It must be remembered that for culturing tubercle bacilli we must protect the growth from sunlight as this will kill the organism. If fluid culture media are inoculated the transferred material should be deposited on the surface. Should the particle sink growth will not occur.

Sputum smears stained by some Romanowsky method or by the hæmatoxylin-eosin stain are best adapted for the study of various cells, and in particular of the eosinophile cells so characteristic of bronchial asthma. In sputum from cancer of the lungs the large vacuolated cells may be found.

When examining the sputum of the bronchopneumonia of influenza the formol fuchsin gives the best results. The influenza bacilli are found in little masses, frequently grouped about small collections of M. tetragenus. The cocci stain a rich purplish-red, while the small influenza bacilli take on a light pink color.

T. B. sputum showing a mixed infection with streptococci or pneumococci or with the influenza bacillus makes for a bad prognosis. M. tetragenus, which often is present when cavities exist, does not seem to be so unfavorable prognostically.

Red cells show up well in specimens stained by the Romanowsky method; if rouleaux formation is marked, it may indicate pulmonary infarction.

In culturing sputum a mucopurulent mass should be washed in sterile water and should then be dropped into a tube of sterile bouillon. With a sterile swab it should be emulsified and successive streaks made along the surface of an agar or glycerine agar plate. In obtaining cultures from influenza sputum, first smear the material thoroughly over a blood-serum slant; then inoculate, by thorough smearing over the surface of successive blood-streaked agar slants, the material on the surface of the blood-serum slant. The platinum loop should be transferred from one slant to another without recharging. The influenza bacillus seems to grow better if the blood-streaked agar slants are prepared just before inoculating with the sputum. All that is necessary is to sterilize an ear, puncture and take up the exuding blood with a large loop. Cultures for tubercule bacilli are impracticable except with antiformin. A guinea-pig should be inoculated.

The blood-stained watery sputum of plague pneumonia should be cultured on

plates of plain agar and 3% salt agar at the same time. An ordinary smear stained with carbol thionin, however, practically makes a diagnosis.

Pneumococci, M. catarrhalis, and Friedländer's bacillus in sputum are best demonstrated by Gram's method of staining.

The distinct capsule staining of the pneumococci in a Gram preparation of sputum from a suspected case of pneumonia is of value in diagnosis.

The finding of the ray fungus (D. bovis) in sputum gives the diagnosis of actinomycosis. Streptothrix infections of lungs have been confused with tuberculosis.

Moulds, especially Aspergilli, may be found in sputum. Species of Mucor, Cryptococcus, and Endomyces have also been reported.

Amœbæ from liver abscess rupturing into the lung may be found. Very important pulmonary infections are those with Paragonimus westermanii. This is recognized by the presence of operculated eggs in the sputum.

A fluke, F. gigantea, was once found in sputum.

Hydatid cysts, either of the lung or of the liver, rupturing into the lung, may be recognized by the presence of echinococcus hooklets. The material is bile-stained if from the liver.

Strongylus apri has been reported once from the lungs and embryos might be found in the sputum. In pulmonary bilharziosis Schistosoma eggs may be found in the sputum.

The test for ALBUMEN IN THE SPUTUM is of value in the diagnosis of pulmonary tuberculosis.

About 10 c.c. of fresh sputum as pure as possible from saliva is mixed with an equal quantity of water and 2 c.c. of a 3% solution of acetic acid to remove mucin. After filtering the filtrate is tested for albumin. The test is obtained also in pneumonia and pleurisy with effusion.

CHAPTER XXVII.

THE URINE.

MATERIAL for staining is best obtained by centifuging the urine, then pouring off the supernatant urine, then draining the mouth of the centrifuge tube against a piece of filter-paper so that we have only the pus sediment to finally remove with a capillary bulb pipette and make smears.

The addition of a loopful of egg albumen or blood serum to about twice that amount of urinary sediment gives better results. (See under Staining Methods.)

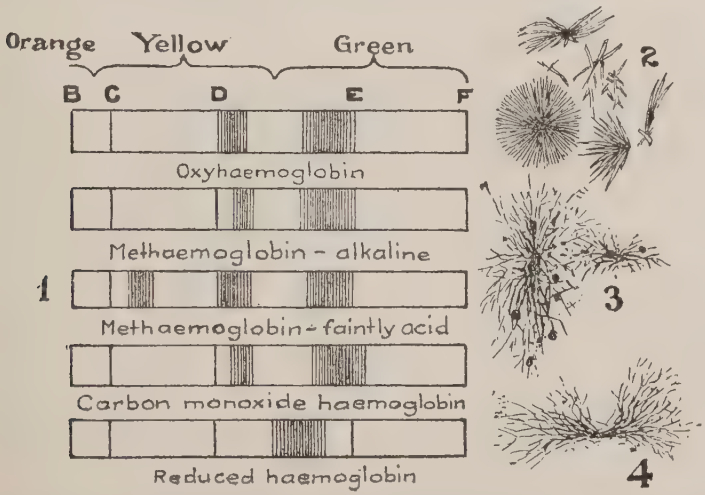


FIG. 98.—1, Various absorption bands of spectrum; 2, crystals of glucosazone (Phenylhydrazine sugar test); 3, Cammidge crystals (interacinar type of pancreatitis); 4, Cammidge crystals (interlobular type of pancreatitis).

The smear may be stained directly by Wright's method or after fixing by heat with Gram's stain, T. B. stain, or hæmatoxylin and eosin. The latter is the best for the staining of epithelial cells and animal parasites; the Gram method for bacteria.

It is frequently difficult to distinguish the spores of moulds from red blood-cells except by measurement and staining reactions. Spores of moulds rarely exceed five microns.

It is difficult to determine the presence of blood in urine in higher dilution than 1 to 300 with the spectroscope. The ordinary occult blood test will show it in much higher dilution.

To secure urine for bacteriological examination catheterization is rarely necessary in men—in the case of women it is the proper method.

The glans penis and meatus should be thoroughly washed with soap and water, after which dilute alcohol (50%) should be used. The greater part of the urine first passed should be rejected and only the last portion passed should be caught in a sterile receptacle. A drop of this urine may be either streaked over the surface of an agar or a lactose litmus agar plate, or so treated after being first diluted in a tube of sterile bouillon.

The lactose litmus agar medium is very useful in distinguishing typhoid or para-

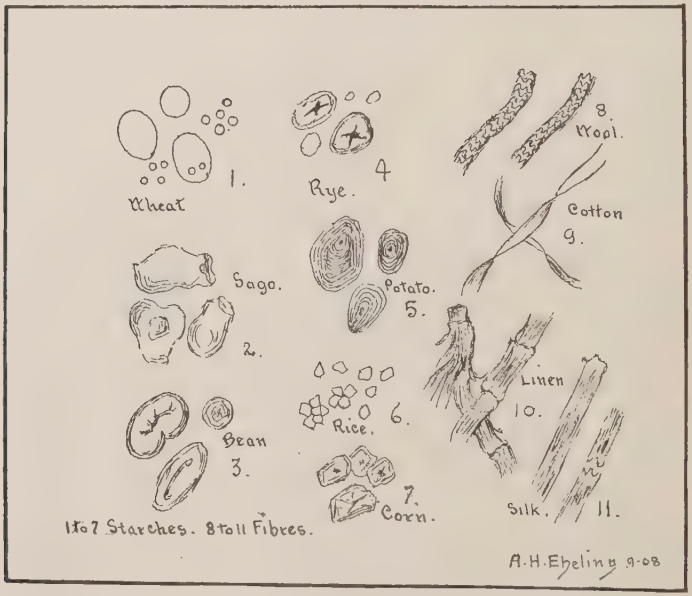


FIG. 99.—Starches and fibers found in urine.

typhoid colonies (blue) from colon, and streptococcus or staphylococcus colonies (pink). The urine may be added to tubes of melted agar and then poured.

The most satisfactory procedure is to deposit one drop on a poured plate and five drops on a second plate. The surface is smeared over with a bent glass rod first smearing out the single drop and then going to the second plate without a second sterilization. Neutral glycerine agar or blood agar is desirable for such organisms as pneumococci or streptococci and, for the gonococcus, Thalman's medium smeared over with a few drops of human serum.

Cystitis from a colon infection gives an acid urine; that caused by Proteus vulgaris an alkaline urine.

The old designation B. termo so often employed in connection with the bacteriology of the urine in older works applied to the proteus group and M. ureæ to ordinary staphylococci.

The bacillus of typhoid and the micrococcus of Malta fever are also found in the urine. This elimination in urine of bacilli by typhoid carriers is of great importance in the spread of the disease.

While the smegma bacillus in urine may be differentiated from the tubercle bacillus by the former losing its red color, by prolonged decolorization with acid alcohol, yet it is chiefly by the subcutaneous inoculation of the guinea-pig that we should diagnose genito-urinary tuberculosis. Inject the sediment after centrifuging.

The method recommended by Gasis which depends on the alkali fast properties of the T. B. has not given me satisfactory results.

Gonococci are reported from Gram-stained smears.

To culture gonococcus material the transfer to culture media should be made almost immediately after obtaining the material from the patient. M. catarrhalis is a rare finding.

Staphylococcus and Streptococcus infections about the mouth as well as such infections in heart or joint may show the presence of the causative organisms in the urine. At times bacterial infections of the kidney may give symptoms of renal stone.

As it is much easier to culture urine than blood a bacteriological examination of the urine may give us the desired information and the organism for the autogenous vaccine. Salt mouth bottles with cotton plugs, when sterilized, make cheap and satisfactory containers. The urine should be plated out as soon as possible after its passage. As a rule when organisms are present in the urine they are in such numbers that the question of contamination rarely arises.

Yeasts and moulds frequently contaminate urine, especially diabetic urine, after it has been passed. Amœbæ and flagellates (Trichomonas vaginalis in females) may be found in urine.

Eggs of Schistosomum hæmatobium (bilharziosis) are important diagnostic findings; these are terminal-spined. Those of rectal bilharziosis are, as a rule, lateral-spined.

In chylous urine the filarial embryos may be found. This examination is facilitated by centrifugalization.

The eggs of the E. gigas may be recognized in urinary sediment by their pitted appearance.

The vinegar eel may be found in the urine of females who have used vaginal douches of vinegar.

Echinococcus hooklets, scolices, or laminated membrane have been found in the urine.

The larval dibothriocephalid, Sparganum mansoni, has been reported three times in urine (urethra).

Oxyuris from the vagina may be found in urine.

Various mites may be found in urinary sediment as the result of lack of care in the washing of the receptacle and are entirely accidental.

Unless having the characteristics of the itch mite and in a person showing scabies lesions about the genital organs the diagnosis of the mite as A. Scabiei should not be made.

Crystals of biliverdin may be found in the urinary sediment in marked jaundice. They somewhat resemble crystals of tyrosin but are brownish in color while those of tyrosin are black. Furthermore, it is excessively rare to find crystals of leucin and tyrosin in the urinary sediments, and in such diseases as acute yellow atrophy of the liver, the urine should be concentrated to one-tenth its volume and the residue treated with alcohol. The tyrosin crystalline sheaves and the leucin striated globules crystallize out from the alcohol.

FIG. 100.—Epithelium from different areas of the urinary tract. *a*, Leukocyte (for comparison); *b*, renal cells; *c*, superficial pelvic cells; *d*, deep pelvic cells; *e*, cells from calices; *f*, cells from ureter; *g*, *g*, *g*, *g*, *g*, squamous epithelium from the bladder; *h*, *h*, neck-of-bladder cells; *i*, epithelium from prostatic urethra; *k*, urethral cells; *l*, *l*, scaly epithelium; *m*, *m'*, cells from seminal passages; *n*, compound granule cells; *o*, fatty renal cell. (*Ogden.*)

URINARY SEDIMENTS.

Turbidity of the urine is most often due either to bacterial contamination, amorphous urates (sedimentum lateritium) or phosphates.

Urates go into solution upon heating and phosphates upon the addition of a few drops of acetic acid.

In turbidity due to bacteria contaminating the urine subsequent to its passage it is best to call for another sample.

To preserve urinary sediments formalin is the best for casts and epithelial cells while for general use one may employ a piece of camphor or the addition of one volume of saturated borax solution to four volumes of urine.

Chloroform does not answer for sediments as it does for urine to be examined chemically. To take up a sediment insert a pipette to the bottom of the tube with the opposite opening closed by a finger, then tease the sediment into the pipette opening in the centrifuge tube, by manipulating the fingers.

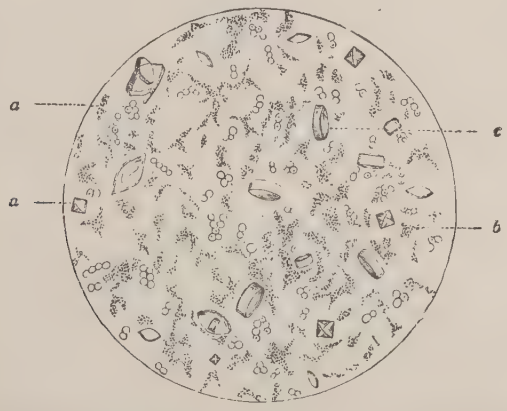


FIG. 101.—Deposit in acid fermentation. a, Fungus; *b*, amorphous sodium urate; *c*, uric acid; *d*, calcium oxalate.

In a urine of acid reaction we may find the following unorganized sediment:

I. Amorphous sodium or potassium urates. Usually yellowish red. Heat and alkali bring about solution.

FIG. 102.—Deposit in ammoniacal fermentation. *a*, Acid ammonium urate; *b*, ammonium magnesium phosphate; *c*, bacteria.

II. Uric acid. Whetstone crystals of yellowish-red color. Soluble in alkalis but not by heat. Abundant sediment of uric acid crystals may be due to too great concentration or too great acidity of the urine rather than to the so-called uric acid diathesis.

III. Calcium oxalate. Octahedral crystals or dumb-bell shapes which are highly refractile. Often due to diet (asparagus, tomatoes, spinach, rhubarb, etc.).

IV. Cystin occurs in six-sided crystals which are soluble in ammonia.

In a urine of alkaline reaction we may expect:

I. Triple phosphates (NH_4 $MgPO_4$). Usually in coffin-lid or fern-like form. Easily soluble in acetic acid.

II. Calcium phosphate and calcium carbonate which effervesce on the addition of acid.

III. Ammonium urate. These show as the thorn-apple structures.

The presence of ammonium urate, particularly if with triple phosphates, denotes bacterial decomposition within the genito-urinary tract provided the urine is just

FIG. 103.—Fatty and waxy casts. *a*, Fatty casts; *b*, waxy casts. (*Greene.*)

passed. Pus cells derived from the site of inflammation should be present also. While certain bacteria might possibly bring on chemical changes without giving rise to inflammation yet such a possibility is so rare as to be negligible. In the presence of amorphous phosphates one should always think of exogenous sources as vegetable diet or withdrawal of proteid food before thinking of disordered metabolism.

Organized Sediment.—An occasional leukocyte may be found in the urine of healthy people. Any abundance of leukocytes indicates inflammation of genito-urinary tract. Some workers count the pus cells in urine by the same technic used for the leukocyte count of the blood. A urine having 100,000 pus cells per c.mm. will give as a result about 0.1% albumin.

Leukocytes are found in abundance at times in the urine of women without

pathological significance. Red blood cells usually show as pale doubly ringed bodies. They appear in inflammations, particularly stone or schistosome infection. They may be found in conditions where toxins are being eliminated through the kidneys, as in tuberculosis. The menstrual period of women must be kept in mind in the examination of urine sediments.

Epithelial Cells.—For morphology of cells from different locations see illustration. It is almost impossible to state positively the origin in the genito-urinary tract of certain cells. Very trustworthy evidence however is finding of epithelial cells on casts or the so-called compound granule cells (fatty degenerated renal epithelium). Sheets of more or less small round or caudate epithelial cells are rather significant of pyelitis. Vaginal epithelium resembles that gotten from scraping the buccal mucosa.

Of casts we have (1) hyaline, narrow and homogenous. They do not prove nephritis. (2) Epithelial casts. Usually indicative of nephritis but very slight inflammatory processes can cause them. (3) Blood casts. (4) Granular casts. If coarse granules rather significant of chronic nephritis. Finely granular casts do not seem to have any more significance than hyaline ones. As a matter of fact under dark ground illumination hyaline casts show a granular structure. (5) Waxy casts are highly refractile, show fissuring of margins and are of serious prognostic import (chronic nephritis).

Cylindroids are drawn out bodies showing tapering ends, irregularity of diameter and longitudinal striations.

It will be found that a 2/3 in. objective gives almost all the information required as to casts. It is quicker and gives more positive information.

Mounting a sediment in Gram's solution or tinging it with the merest trace of neutral red is of much assistance.

	Amount and character of urine	Sp. gr.	Albumin	Sediment	Etiology	Special features
Acute nephritis....	Diminished	High 1025 to 1030.	High 0.5% and more.	Large amount. Hyaline granular epithelial and blood casts. Renal epithelium. Red and white blood-cells.	Infectious diseases. Chilling. Poisons.	Sudden onset. Œdema often marked, especially of face. Mild or even severe uræmic symptoms. Pulse tension increased but heart not hypertrophied.
Ch. Parenchym. Nephritis.	Normal or diminished.	Moderately high 1020 to 1025.	Large amount. 0.5 to 2%.	Abundant. All kinds of casts including fatty and waxy. Red blood cells. Much fatty epithelium.	Following acute attack. Alcohol, syphilis, malaria, T. B. suppuration.	Marked œdema. Uræmia common. Hypertrophied left ventricle. Blood pressure increased.
Ch. Interstitial Nephritis.	Greatly increased (2000 to 4000 c.c.). Bright color.	Low 1005 to 1010.	Trace; rarely exceeding 0.1%.	Very slight. Very few hyaline and granular casts, Red and white blood-cells.	Heredity, gout, syphilis, alcohol, lead, arteriosclerosis.	No œdema until later. High blood pressure (200 to 250). Cardiac hypertrophy; often uræmia and albuminuric retinitis.
Passive Congestion.	Diminished; high color.	High 1025 to 1030.	Small amount.	Sedimentum lateritium; occasional hyaline casts. Red and white blood cells. Renal epithelium exceptional.	Ch. heart and lung disease.	No uræmia. Symptoms attributable to heart.
Pyelitis.	Normal, slight turbidity. Often hæmaturia.	Normal.	Slight.	Abundance of pus cells. Caudate epithelium often in clusters. At times red blood-cells.	Bacterial infection ascending or descending. Stone.	Reaction of urine acid. No tenesmus.
Cystitis.	Normal but very turbid.	Normal.	Slight.	Very great abundance of pus cells. Much mucus and bacteria.	Bacterial infection.	Reaction of urine alkaline or very faintly acid. Tenesmus.

CHAPTER XXVIII.

THE FÆCES.

It is advisable to examine a stool macroscopically before taking up the miscroscopical examination. The mucus shreds or casts of the bowel in mucous colitis or membranous enteritis may give the diagnosis of obscure abdominal pain. Pus in stools may often be noted without the aid of the miscroscope.

The normal stool is sausage shaped and soft. Neither the special form of scybalous masses called sheep pellets nor the pencil-like nor the tape-like excrement prove the existence of stricture of the intestinal lumen although suggestive of such a condition. The mucus of bacillary dysentery is opaque and grayish from the great number of pus and phagocytic cells. It is well to remember that Charcot Leyden crystals, which are practically always absent from bacillary dysentery stools, are not infrequent findings in the amœbæ containing stools; of course, these crystals appear in other intestinal parasite infections.

In obstruction of the common bile duct we have acholic, whitish, foul-smelling stools. If the putty color be due to bacterial change exposure to the air will restore the brownish tinge.

Sprue stools are white-wash to putty colored, pultaceous, and filled with air bubbles. The amount is excessive.

Fatty stools are best examined microscopically.

As so many solid masses resemble gall-stones it is well to dissolve the suspected mass in hot alcohol and examine for cholesterin crystals upon evaporation of the alcohol.

If the fæcal examination is to be made for the diagnosis of amœbæ, in a case where the characteristic mucus stools are not present, or to verify the existence of flagellates, it is best to give a dose of salts early in the morning and examine the liquid stools which follow such treatment. This treatment is satisfactory for examination for intestinal parasites or ova.

A very practical way of obtaining amœbæ is to pass a rectal tube or a piece of drainage tube with fenestrations into the bowel, and amœbæ may be found in the mucus filling the perforations in the tube.

If the purpose of the examination is to determine the digestive power of the alimentary tract for proteids, carbohydrates, or fats, it is best to use a test diet, as that of Schmidt and Strasburger.

Prior to using this test diet, one should familiarize himself with the macroscopic and microscopic appearances resulting from such a diet in a normal person; information is then at hand to judge of variations from the normal. The examination of the fæces of persons, on ordinary and specifically undetermined articles of diet, is very unsatisfactory when the state of digestion of muscle fibers and the question of fat digestion are at issue.

In examining the fæces of the normal person and likewise with the patient, wait until the second or third day so that the fæces of previous diets may have passed out. A charcoal powder taken before commencing the diet serves as an indicator.

Diet: breakfast, 7 A. M., bowl of oatmeal gruel (40 grams oatmeal, 10 grams butter, 200 c.c. milk, 300 c.c. water). Also one very soft-boiled egg (1 min.) and 50 grams zwieback. In the forenoon, 500 c.c. of milk.

For dinner, 2 o'clock, chopped beef broiled very rare (125 grams with 20 grams butter poured over it). Also a potato puree (200 grams mashed potato, 50 grams milk, 10 grams butter). Also 1/2 liter of milk and 50 grams zwieback.

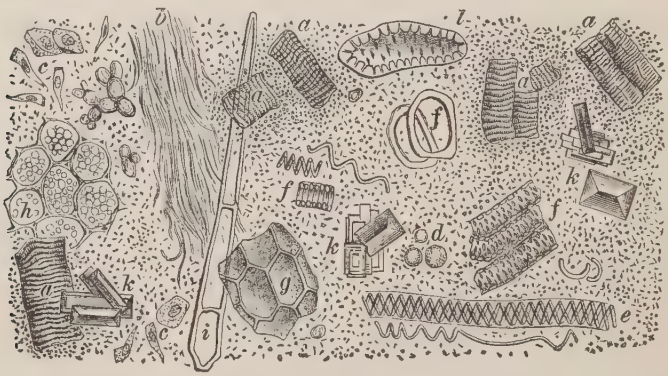


FIG. 104.—Microscopical constituents of fæces. (*v. Jaksch.*) *a*, Muscle fibers; *b*, connective tissue; *c*, epithelium; *d*, leukocytes; *e*, spiral cells; *f*, *g*, *h*, *i*, various vegetable cells; *k*, "triple phosphate" crystals; *l*, woody vegetable cells; the whole interspersed with innumerable microorganisms of various kinds.

For supper, 7 o'clock, the same articles as for breakfast.

This detailed diet may be varied to suit circumstances as regards interchanging meals. Furthermore, the milk may be taken in the form of tea or cocoa or cooked with the other food. Even a small amount of wine may be permitted. The diet taken, however, should absolutely conform to the following requirements: 1. the taking of 1/4 pound chopped beef, a portion of which should be half raw; 2. the milk taken should amount to about a quart; 3. about 4 ounces of bread or toast and from 4 to 8 ounces of potato puree should be eaten daily.

The detailed diet contains about 110 grams albumin, 105 grams fat and 200 grams carbohydrates with a fuel value of 2247 calories.

The stool is best collected in quart fruit jars and examined as soon after evacuation as possible. The wooden spatula like tongue depressors are well adapted to handling the specimen.

Having familiarized one's self with the degree of digestion of muscle, starch, and fat in a normal person, we are in a position to judge of the state of assimilation in a patient.

The first part of the test is the macroscopical one. For this grind up a fæcal mass of 1/2 to 1 inch diameter in a mortar, gradually adding water until it has the consistence of a broth. About 1/2 c.c. of this emulsion should now be squeezed out between two slides and studied against a dark surface and then when held up to the light. The normal stool gives a rather uniform brownish homogeneous layer. Connective-tissue remnants (indicative of gastric derangement) show as whitish fibers. Undigested muscle tissue remnants as reddish-brown splotches. Fat particles as whitish-yellow clumps. Potato remnants appear like sago grains and mash out easily like mucus. Mucus is best noted in the fæcal mass before making the emulsion. In the microscopical test of this emulsion.

We judge of muscle digestion by the intactness of the striations. If a muscle remnant is only a homogeneous yellowish particle, it shows satisfactory digestion. If it is rectangular, with well-defined cross striations, it shows poor digestion for meat (Azotorrhœa). A loopful of fæces should be smeared into a drop of Gram's solution for starch-digestion determination. Normally there should be no blue-staining starch granules.

Soaps are gnarled bodies everted like the pinna of an ear, while soap crystals are comparatively coarse and do not melt on application of gentle heat as do the more delicate fatty acid crystals. Neutral fat is in round or irregular globules. The best stain for fat is Sudan III (saturated solution of Sudan III in equal parts of 70% alcohol and acetone).

Mix up the fæces with dilute alcohol (50 to 70%) and then add a drop of the above solution and apply a cover-glass quickly. The fat globules show as orange or golden-yellow bodies.

By rubbing up a small portion of the fæces in 36% acetic acid, applying a cover-glass and heating over a flame until the preparation shows bubbles, we convert the soaps and other fat combinations into free fatty acids which show as more or less numerous highly refractile bodies which show a crystalline structure as the preparation cools. By practice one learns the amount of such globules to expect with different fat contents in stools.

Steatorrhœa, or the presence of fat in abnormal quantities in the fæces, is shown by the pale, bulky, greasy stools as well as in the microscopical examination.

Average for normals in 1 gm. dried fæces:

Total fat,	225 mg.	(22.5%)
Total fatty acid,	86 mg.	(37.9% of all fat)
Total soap,	74.7 mg.	(33.4% of all fat)
Total neutral fat,	64.4 mg.	(28.5% of all fat)

In normal cases the only fat elements recognizable are yellow calcium or colorless soaps.

As quantity of fat increases (as say 500 to 600 mg.) droplets of neutral fat appear with or without increase in number of soap masses. Also needles and splinters of fatty acid and soaps appear. Much connective-tissue débris shows defect in gastric digestion, as only the stomach digests connective tissue.

A test for activity of fermentation should be made by using a Schmidt apparatus.

A distinct evolution of gas in twelve hours shows starch digestion defect. Such fæces are acid. A delayed production of gas (after twenty-four hours) shows albumin decomposition. Such fæces show an alkaline reaction. The apparatus is shown in Fig. 7. Into a stocky salt mouth bottle we put approximately 5 grams of fæces which have been rubbed up into an emulsion with water and fill the bottle with water. The remaining portion of the apparatus consists of a test-tube or a graduated cylinder fitted with a doubly perforated rubber stopper. One U-shaped glass tube passing through this stopper connects with a second test-tube. This tube serves as a receptacle for any water which may come over from the water-filled tube or graduated cylinder and has an opening punched out of the bottom of the test-tube. The other opening in the twice perforated cork admits a straight tube which connects with a large rubber stopper which fits into the bottle for the fæces. To prepare, fill the graduated cylinder, then push in the doubly perforated cork which is connected with the side receiving tube and the large rubber cork. This latter is then pushed down to fit tightly into the bottle filled full with the fæces emulsion.

In addition to the fæces examination we should check the results from the test diet with indican and nitrogen partition determinations of the twenty-four hour urine specimen—the ratio of ammonia nitrogen to total nitrogen indicating the functional power of liver and the indican the question of stasis in lower part of small intestine.

The most satisfactory test for bile in the fæces is to emulsify a small particle of fæces in a saturated aqueous solution of bichloride of mercury, preferably with a wooden tooth pick, on a concave glass slide. After one or more hours hydrobilirubin-containing fæces show a salmon pink color and bilirubin ones a green color. One should familiarize himself with these reactions in normal cases.

In examining a liquid stool after salts, it is well to color the drop of fæces, which is to be covered with the cover-glass, with a small loopful of 1/2% solution of neutral red. If diluting fluid is used, it should be salt solution, and not water. The neutral red tinges the granules of the endoplasm of amœbæ and flagellates a very striking rose pink color, thus differentiating them from vegetable cells or body cells.

Whether examining the thin fæces or the mucus particle, it is well to reserve report on amœbæ or flagellates until motion is observed. Encysted protozoa are difficult to diagnose.

When a smear preparation is desired, we may smear out a fragment of mucus and stain by Romanowsky's or Gram's method. The character of the bacteria

present appears to be of diagnostic value—especially in the case of infants and young children. Beautiful preparations may be made by mixing the fæces with water, then centrifuging for one minute. This throws down vegetable débris and crystals. Now decant the supernatant fluid, which holds the bacteria in suspension, and add an equal amount of alcohol. Again centrifuge, decant, and smear out and examine the bacterial sediment.

Simply taking a small mass of fæces and emulsifying it with a wooden toothpick on a concave slide in 70% alcohol—then, after the sediment settles, taking up a loopful with platinum loop from the surface and smearing out, gives a very satisfactory smear. Gram's method, with dilute carbol fuchsin counterstaining, gives the best picture.

The Boas-Oppler bacillus may be found in the stools in this way. Normally, a Gram-stained stool shows a great preponderance of Gram negative bacilli and such a finding in a measure excludes cancer of the stomach. Organisms which are Gram positive as well as the Boas-Oppler bacillus are, 1. Lactic acid bacilli—these show Gram negative areas in the slender bacilli. 2. A type of bacillus similar in size to the colon bacillus but Gram positive and noncultivable (found in acid stools). 3. Bacilli of the B. subtilis type.

It is very important to examine the fæces for T. B. With children a diagnosis of tuberculosis may be made in this way when the sputum cannot be obtained, the pulmonary secretion being swallowed. The preparation on the concave slide as described above should be stained for T. B.

To culture for typhoid, dysentery, cholera, or other bacteria, take up the material in a tube of sterile bouillon and smear it out with a swab over a lactose litmus agar plate or an Endo or Conradi-Drigalski plate. Before streaking the plates they should be very dry on the surface. This can be best done by pouring into a plate with a circular piece of filter-paper in the lid and placing in the incubator for one-half hour to dry. The filter-paper absorbs the moisture. Then inoculate the surface of the plate with the fæcal material.

In summer complaints of infants and children the organisms concerned are as a rule related to various dysentery strains of bacilli. Kendall in 293 stool examinations found the gas bacillus (B. ærog. capsul.) in 22 cases. The gas bacillus produces intestinal disorders which are not benefited by lactose but by buttermilk (lactic acid bacteria). For diagnosis, a loopful of the fæces is emulsified in a tube of sterile milk or litmus milk. The emulsion is heated to 80° C. and held at this temperature for 20 minutes. After incubation for 18 to 24 hours, preferably anaerobically, we get (1) a shreddy disruption of the casein, (2) the smell of rancid butter and (3) fully 80% of the casein is dissolved. Smears show short thick Gram positive rods with slightly rounded ends. B. Subtilis is sometimes found but does not give a rancid odor nor the strong disruption of the clot.

It was until recently thought that Cammidge's reaction (urine) when associated with azotorrhœa and steatorrhœa made for a diagnosis of chronic pancreatitis. At present very little importance is attached to the Cammidge reaction.

Loss of weight, anæmia, diarrhœa and pains in the upper abdomen are important indications of pancreatic trouble. As chronic pancreatitis is often associated with cholelithiasis jaundice is frequently present. Glycosuria is not often present. While functional tests are important they do not make for a sure diagnosis.

Müller's method for pancreatic functioning determination is to give a calomel purge two hours after a meal. A little of the liquid stool is smeared on the surface of blood-serum and the tube incubated at 60° C. (paraffin oven). If the surface is smooth, no trypsin was present; if dotted with spots of digestion liquefaction, it shows that the pancreatic secretion is present.

In Schmidt's nucleus test small cubes of beef are hardened in absolute alcohol and then tied up in tiny silk bags. These are recovered from the fæces and sectioned. Complete preservation of nuclei indicates a total absence of pancreatic functioning provided the passage of the tissue be not too rapid as by diarrhœa.

Epithelial cells are generally more or less disintegrated. In the mucus of bacillary dysenteric stools, however, large intact phagocytic cells are frequent, which may be mistaken for encysted amœbæ.

Triple phosphate crystals are frequently observed in fæces, as may also be crystals of various calcium salts. Charcot-Leyden crystals are rather indicative of helminthiases.

Various flagellates, and in particular Lamblia, may be responsible for diarrhœal conditions which may cause rather serious symptoms.

Balantidium coli has been reported several times as the cause of dysenteric conditions. Coccidiadea are found in the fæces.

It is in the fæces we examine either for the parasites or for their ova in connection with practically all the flukes, except the lung fluke and the bladder fluke; for intestinal tæniases and for practically all the round worms, except the filarial ones.

Bass has recommended that fæces which have been made fluid be centrifuged and the supernatant fluid containing vegetable débris be poured off. The sediment contains hookworm eggs. Then pour on sediment a calcium chloride solution of sp. gr. 1050. Again centrifuge and decant. Next add calcium chloride solution of a sp. gr. of 1250 and centrifuge. This brings to the surface the hookworm eggs which may be pipetted off. As a rule, the finding of hookworm eggs is very easy without such a technic.

In the tropics, the examination of the fæces vastly exceeds in value that of urine and is possibly more important than blood examinations.

The larvæ of various insects may at times be detected in the stools, as well as certain acarines (cheese mites, etc.).

The test for occult blood is indicated in helminthiases as well as in the conditions for which it is usually tested.

CHAPTER XXIX.

BLOOD CULTURES AND BLOOD PARASITES.

Clinically, the most important examinations of the blood for parasites is for the presence of various bacterial infections and for certain blood protozoa and also filarial embryos.

The modern method of culturing blood, especially for the detection of typhoid or paratyphoid bacilli, is by the use of the bile media of Conradi. Test-tubes are filled with 7 to 10 c.c. of 1% peptone ox bile, or ox bile alone, and the medium is sterilized in the autoclave. It is good practice to place the syringe in a plugged test-tube containing salt solution, with the needle unscrewed. After autoclaving, the sterile syringe can be taken to the bedside in the test-tube. Using a wide test-tube, a forceps can be sterilized at the same time and used to attach the needle to the barrel of the syringe.

By using a piece of glass tubing into which the needle is inserted we may sterilize the syringe easily in the test-tube. The glass tubing prevents the steel needle from coming in contact with the glass of the test-tube and so prevents cracking the test-tube. Instead of a syringe a better apparatus is a test-tube or an Erlenmeyer flask with a double perforation stopper for insertion of two pieces of glass tubing, one joined to a piece of rubber tubing carrying the needle and the other attached to a rubber tube for suction by the operator's mouth. See Fig. 7.

The skin should be scrubbed gently with green-soap solution and water for about three minutes. The skin of the area to be punctured should then be sterilized by the gentle application of Harrington's solution (not scrubbed) for one-half minute, and should then be washed with sterile water. It appears to be safe to simply scrub the area with 70% alcohol for one or two minutes. Applications of pure carbolic acid on a gauze wad for a few seconds followed by neutralization with 70% alcohol gives satisfactory sterilization. The present method of sterilizing skin for taking blood or inoculating vaccines is simply to smear the site of entrance for the needle rather heavily with tincture of iodine. A tourniquet is now applied to distend the vein, and the needle is inserted in the direction of the venous flow. Withdrawing 5 to 10 c.c. of blood, we loosen the tourniquet, (otherwise the blood may flow from the puncture) then withdraw the needle, and force out about 1/2 c.c. into the first bile tube, about 1 c.c. into the second, and 2 or 3 c.c. into the third. It is well to reserve some of the blood for Widal tests.

The bile tubes are now incubated for ten to twelve hours and then transfers are made to bouillon tubes. These bouillon tubes can be used in six to eight hours for testing the organism against known typhoid or paratyphoid sera. Test-tubes containing 10 c.c. of ordinary bouillon with 1% of sodium citrate are as satisfactory as bile media.

Some prefer a 2% sodium glycocholate in bouillon while others use a 2% solution of ammonium oxalate in bouillon for blood cultures.

Some prefer to streak plates of lactose litmus agar with material from the bile tubes instead of inoculating the bouillon tubes. Contamination with staphylococci or the presence of staphylococci, streptococci, or plague bacilli in septicæmic conditions show easily accessible colonies.

Schotmuller adds 1 to 3 c.c. of blood to liquefied agar at 45° C., and after mixing pours into plates. The standard method formerly was to add the blood to an excess of bouillon (1 to 5 c.c. of blood to 100 c.c. or more of bouillon).

A very useful procedure in the isolation of streptococci, pneumococci, plague and anthrax bacilli is to inject 1 to 2 c.c. of blood into suitable animals. When injecting mice use only about 0.2 c.c.

By using the bile media, we can take the blood from the ear in typhoid cases, if preferred. Then if chance staphylococcic contamination occurs, such colonies are readily differentiated from typhoid ones by the pink color on lactose litmus agar. For culturing blood in septicæmic conditions, the blood should always be drawn from the vein and cultured either by mixing 1 to 2 c.c. with melted agar and then pouring plates or by transferring to bouillon in excess (at least ten times as much bouillon as blood) and after eighteen to twenty-four hours' incubation plating out. For streptococcus and pneumococcus blood agar plates are to be preferred, the pneumococcus giving green colonies with only a suggestion of hæmolysis while the streptococcus gives an opaque colony with a distinct hæmolytic zone surrounding it.

Typhoid cultures are best obtained in the first week of the disease, after that time the Widal is the test of preference.

If a paratyphoid serum is not at hand for testing, it may suffice to inoculate a glucose bouillon tube or a Russell lactose glucose litmus slant; gas production indicates paratyphoid. This test should be applied when a very motile organism does not show agglutination with a known typhoid serum. Anthrax and glanders should be considered in blood cultures.

In Malta fever it must be remembered that colonies do not show themselves for several days. Addition of blood to melted agar is a good procedure.

Blood for culturing typhoid or the paratyphoids may be taken with a Wright's tube from the ear or finger. Dipping the hand in hot water assists the flow of blood. The supernatant serum after centrifugalization should be pipetted off with a sterile pipette and reserved for agglutination tests while the clot is dropped into a bile tube. (Clot culture.)

Rosenberger was the first to insist upon the importance of examination of blood for T. B. Brem considered that many cases of finding of acid-fast bacilli were not of T. B. The Kurashigi-Schnitter method for tubercle bacilli in blood is to take

about 1 c.c. blood and put in a centrifuge tube containing 5 c.c. of 3% acetic acid. After the red cells are thoroughly laked centrifuge, pipette off supernatant fluid and dissolve the sediment in 5 c.c. antiformin. When dissolved add 5 c.c. absolute alcohol and centrifugalize for twenty minutes. Smear out the sediment and stain.

The examination of the blood for the parasites of malaria, filariases, kala-azar and spirillum fevers has been discussed under their respective headings.

With trypanosomes from human trypanosomiasis, smears from gland juice or cerebrospinal fluid seem more satisfactory to examine than blood smears unless the blood is taken in 5 to 10 c.c. quantities and centrifuged in sodium citrate salt solution.

The latest method in the diagnosis of trichinosis is to take 5 to 10 c.c. of blood from a vein at the time of the migration of the embryos to the muscles (10 to 20 days). This is forced out into a centrifuge tube containing 3% acetic acid, and the sediment examined for trichina larvæ.

CHAPTER XXX.

THE STOMACH CONTENTS.

From a microscopical standpoint there is comparatively little that is of value in the examination of the gastric contents; there is nothing very specific about the findings.

A test meal is not a necessity as in the chemical examination, but either vomitus or material withdrawn with a stomach-tube two or more hours after an ordinary meal suffice.

The most satisfactory specimen is one taken before the giving of the test meal.

The washings from the stomach are allowed to stand until the sediment has fallen to the bottom and an examination of this is made.

The microscopical diagnostic points in connection with distinguishing cancer of the stomach from nonmalignant dilatation are: 1. Fragments of cancer tissue. These are very rarely found and are most difficult to diagnose. 2. The presence of flagellates in the early stages of cancer (the so-called anacid stage preceding the development of lactic acid). As flagellates prefer an alkaline medium, they disappear after the acidity due to lactic acid comes on. 3. The presence of the Boas-Oppler bacillus. There are probably several organisms so designated. They are lactic acid producers and are characterized by being very large bacilli ($7 \times 1\mu$) and arranged in long chains which stretch across the field of the microscope. They are Gram positive and do not form spores. They can be cultivated on media rich in blood and are aerobic. They should only be reported when present in great abundance and in long chains. Heinemann thinks it probable that the Boas-Oppler bacillus, Leptothrix buccalis, and B. bifidus may be identical with B. bulgaricus (see under Milk). 4. The absence of sarcinæ and yeasts. The presence of these sarcinæ and fungi in vomitus is indicative of a simple dilatation.

In chronic gastritis the picture of mucus entangling large numbers of epithelial cells is characteristic.

In examining the sediment from the filter-paper after filtering off the stomach contents always use a dilute Gram solution (about 1 to 4) for mounting the sediment. Muscle fibers, yeast cells, red blood-cells, and epithelial cells are stained a golden yellow. Starch granules are stained blue while fats are unstained and show as globules of varying sizes.

CHAPTER XXXI.

EXAMINATION OF PUS.

Pus may be collected for examination either 1. with a platinum loop, 2. with a sterile swab, 3. with a bacteriological pipette or 4. with a hypodermic syringe.

It is always well to make a smear and stain it by Gram's method at the same time that cultures are made. The Gram stain gives information as to the abundance of organisms in the pus and as to the probable findings in the culture. Pneumococci and streptococci are differentiated from the staphylococci in this way without the necessity of more or less extended cultural methods.

Smears from material examined for gonococci may show Gram negative diplococci which, however, do not generally have the morphology of the gonococcus. They are furthermore extracellular.

The M. catarrhalis has been reported from urethral smears though very rarely. Diphtheroid organisms are not uncommon. Gram positive cocci are rather common in smears from discharges of chronic gonorrhœa.

When autogenous vaccines are to be made, the isolation of the exciting organism is necessary. This is best done by streaking the pus, taken up with a sterile swab and emulsified in a tube of bouillon, over the surface of an agar plate. Practically as convenient and providing a more nutritious medium is to smear the material on a loop or swab over the surface of a blood-serum slant, then to inoculate a second tube from the first without recharging the loop or swab, and so on until three or four tubes are inoculated. Isolated colonies should be obtained in a third or fourth tube.

In examining blood-serum slants inoculated with purulent material, always examine the water of condensation for streptococci.

A bacteriological pipette is very useful when pus is to be sent to a laboratory; the tip can be sealed in a flame and the cotton plug at the other end insures the noncontamination of the contents. The material may be drawn up either with the mouth or with a rubber bulb.

The hypodermic syringe is very useful in puncturing buboes, etc., especially in plague. A small pledget of cotton on a toothpick dipped into pure carbolic acid and touched to a spot over the bubo, which after about thirty seconds is soaked with alcohol, makes a sterile anæsthetic spot at which to introduce the needle of the syringe. It must be remem-

bered that when plague buboes begin to soften, the plague bacilli may be replaced by ordinary pus organisms.

It is remarkable how frequently we get pure cultures from abscess material. In purulent material from abdominal abscesses we are apt to obtain mixed cultures, especially the colon bacillus and B. pyocyaneus, in addition to ordinary pus organisms.

When it is a question between streptococci and pneumococci, it is well to inoculate a mouse; the capsulated pneumococci at the autopsy make the diagnosis.

Animal inoculation is also necessary in plague and glanders, and possibly anthrax. When tetanus is suspected, it should be examined for as described under Tetanus. Tuberculosis should also be identified by inoculating a guinea-pig, as well as by acid-fast staining and culture, if there is any doubt as to the nature of the material.

The black or yellow granules of madura foot, as well as those of actinomycosis, should be examined as recommended in the section on fungi.

Amœbæ, coccidia, and larval echinococci may be found in purulent material, as may also various other animal parasites, as fly larvæ, sarcopsyllæ, etc.

The pus from an amœbic abscess of the liver is as a rule sterile when cultured.

The examination at the time of operation or exploration frequently shows an absence of amœbæ as well as of bacteria. Two or three days later amœbæ may be found in the pus draining from the abscess cavity.

Flukes, round-worms, and whip-worms may as a result of their wandering from the intestinal lumen cause abscesses.

Serious ulcerations may follow infection with the Guinea-worm.

CHAPTER XXXII.

SKIN INFECTIONS.

CULTURAL methods are as a rule to be preferred in the bacteriological examination of the skin.

This is best done by washing the surface to be examined with soap and water, in order to eliminate chance organisms which may have settled on the surface of the skin in dust or as a result of contact with material containing them. Scrapings are then made with a sterile dull scalpel, and this material is emulsified in a drop of sterile water in the center of a Petri dish. A tube of melted agar at 42° C. is then poured on the inoculated drop and, by mixing, the bacterial flora is distributed over the entire surface of the plate. Of the colonies developing on such plates probably 80% will be found to be staphylococci, and of these the greater proportion will be staphylococci showing white colonies.

Occasionally the aureus or citreus may be isolated.

Streptococci and colon bacilli are rarely found.

The Staphylococcus pyogenes aureus is the organism usually isolated from furuncles, circumscribed abscesses, and carbuncles.

Streptococci are the organisms to be expected in phlegmonous infections.

Cold abscesses, which are frequently due to tuberculous infection, are, as a rule, sterile.

Acne pustules may show staphylococci or the microbacillus of acne may be present.

The Bacillus acnes is a short broad bacillus often showing a beaded appearance when stained by Gram's method. It is Gram positive According to Hartwell it grows readily on glucose agar when cultivated anaerobically (Wright's method). Colonies appear in four to five days.

Sabouraud's medium for its culture is: Peptone 20 grams, glycerine 20 grams, glacial acetic acid 5 drops, agar 15 grams and water 1000 c.c. The bottle bacillus, which morphologically resembles a yeast, is considered to be the cause of dry pityriasis capitis. It may also be found in the comedones of children.

In the tropics, an organism which at times produces lesions similar to impetigo and again pemphigoid eruptions and at other times wide-spreading erysipelatous conditions gives cultural characteristics similar to S. pyogenes aureus. It is probably only a virulent aureus. It has been described under the name of Diplococcus pemphigi contagiosi.

The Staphylococcus epidermidis albus, or stitch abscess coccus, is considered by Sabouraud to be the cause of eczema seborrhoicum.

It is in scrapings from the skin of lepromata that we find acid-fast organisms in the greatest profusion. In tuberculosis of the skin the tubercle bacilli are exceedingly scarce. Inoculation of a guinea-pig will probably give positive results with the tubercle bacillus. The leprosy bacillus is noninoculable for experimental animals.

Anthrax and glanders cause skin lesions which can only be surely diagnosed culturally or by animal inoculation.

Plague bacilli may be isolated from the primary vesicles appearing at the site of the flea bite.

Tropical phagedæna is thought by some to be due to a sort of diphtheroid organism. The organisms of Vincent's angina may cause tropical ulcer.

The skin diseases due to fungi are discussed under that section. Of the skin affections caused by animal parasites, ground itch is the most important. This is a form of dermatitis due to the irritation set up by the hook-worm larvæ penetrating the skin of the foot and leg.

The Sarcopsylla penetrans or jigger (sand flea) is an important agent in ulcerations about the foot.

Certain acarines cause skin lesions, as is also the case with the larvæ of certain flies.

The itch mite (Sarcoptes scabiei) is an important animal parasite of the skin.

The various lice, fleas and bed bugs are well understood as causes of skin irritation.

Filarial infections are also important especially the ulcers of the Guinea-worm, Calabar swellings of F. loa, the cystic tumors of F. volvulus and the varicose groin glands and elephantiasis of F. bancrofti.

Leeches, as H. ceylonica, may cause serious ulceration.

Oxyuris may cause a severe irritation about the region of the groin and inner surfaces of the thigh, and especially about the vulvar region of female children.

Gnathostomum siamense, a nematode with two lip-like structures and spine-like structures covering its anterior one-third, has been found once in a tumefaction of the breast.

Plerocercoid larvæ of Dibothriocephalidæ have been found in the subcutaneous tissues.

Certain skin diseases, as Oriental sore and yaws, are protozoal in origin.

CHAPTER XXXIII.

CYTODIAGNOSIS.

THIS method of diagnosis is chiefly employed in the examination of cellular sediments of pleural, ascitic, and cerebrospinal fluid.

The fluids which pathologically collect in the serous cavities are divided into two classes, 1. the transudates, which form as the result of some circulatory inadequacy and 2. the exudates, which result from inflammatory processes.

Transudates have little or no fibrin and very few cellular elements and do not contain nucleo-albumin. Exudates contain nucleo-albumin and usually have a specific gravity above 1018, while that of the transudates is lower than 1018.

There are two simple methods for differentiating transudates and exudates. Moritz adds 2 drops of a 5% solution of acetic acid to the fluid to be tested. A heavy, cloud-like precipitate shows the fluid to be of inflammatory origin (an exudate). A transudate may produce a slight opalescence. Rivalta's test consists in dropping a drop of the fluid to be tested into a cylinder containing 2 drops of glacial acetic acid in 100 c.c. distilled water. A nebulous cloud as the drop of fluid sinks shows an exudate.

For pleural fluids we should receive the material in centrifuge tubes about one-fourth filled with 2% sodium citrate salt solution. This prevents clotting. Having thrown down the sediment, the supernatant fluid is poured off, and in its place a 1% aqueous solution of formalin is added. After mixing and allowing to stand for about five minutes, centrifugalization is again repeated and, pouring off the supernatant formalin solution, we make smears from the sediment. This is either stained by a Romanowsky method or, after fixing with heat (burning alcohol), the smear is stained with hæmatoxylin and eosin.

With ascitic fluid it is usually sufficient to centrifuge the fluid, then decant off the supernatant fluid and drain by means of a piece of filter-paper held at the mouth of the upturned tube. The sediment adheres to the bottom of the tube and is best emulsified with the small amount of fluid remaining by means of a bulb pipette. The material is sucked up, smeared out on a slide with a second slide as for blood and stained preferably with Giemsa after fixation. H. E. staining brings out mitotic figures best. If the fluid has coagulated it is best to take a little of the coagulum and stain it with neutral red as for vital staining. It is difficult to dissociate the cells from the clot. The wet Giemsa method described for blood gives good results with puncture fluid sediments.

At the time of securing fluid for cytodiagnosis, cultures should be made on blood-

serum for various pyogenic bacteria and, if tuberculosis is suspected, inoculation of a guinea-pig is indicated.

The interpretation of cellular sediments is more difficult than many books would indicate, there being many factors which tend to complicate the findings.

The polymorphonuclears in purulent fluids often show fatty degeneration, swollen and faintly staining nucleus or a breaking up of the nucleus into small deeply staining masses (nuclear fragmentation). Such fragments in the smear may be confusing. The endothelial cells often show fatty degeneration in the cytoplasm and we often note bacteria and other cells which have been phagocytized by them. Where proliferation of endothelial cells is going on actively the cells show a rather deeply staining cytoplasm as compared with the light staining cytoplasm of the cells in transudates.

The following are the leading differentiations:

1. A smear showing almost entirely lymphocytes with a few red cells and very rarely a polymorphonuclear indicates a tuberculous process.

2. Where a pyogenic process is engrafted on a tuberculous one, we have still the red cells, some degenerated lymphocytes, and in particular polymorphonuclears showing fragmentation of their nuclei.

3. When a hydrothorax results from chronic heart or kidney disease, the characteristic cell is the endothelial cell, which greatly resembles a large mononuclear. These cells often are arranged in plaques.

4. Some authorities consider that the cancer cell can be recognized by its occurring in masses and having a markedly vacuolated cytoplasm. It has been claimed that they contain glycogen by which means we can distinguish them from endothelial cells which they so much resemble. If such cells should show mitosis the finding would be suggestive. For mitotic figures wet fixation with some bichloride fixative, with H. E. staining, is best.

Jousset introduced inoscopy as a means of diagnosing tuberculosis. The fluid was allowed to coagulate and was then digested with an artificial gastric juice. The digested material was then centrifuged and the sediment examined for tubercle bacilli. This process does not seem to have met with much favor in this country. (Using sodium citrate obviates the necessity for digesting the coagulum.)

The same points will hold for ascitic fluid as for pleural fluid.

In taking cerebrospinal fluid for culture and cytodiagnosis we use a stout antitoxin needle without attaching a syringe. Aspiration is responsible for many of the ill effects of lumbar puncture. The needle should be about 4 inches long for an adult. Sterilize the skin and needle as described for blood cultures from a vein. To make a lumbar puncture, place patient on left side with knees drawn up. A line at the level of the iliac crests passes between the third and fourth lumbar vertebræ. Select a point midway between the spinous processes of these lumbar vertebræ and

enter the needle 2/5 of an inch to the right of this point, pushing the needle inward and upward. Collect the material in a sterile test-tube. Make cultures on blood-serum and then centrifugalize and examine the sediment as for pleural fluids.

In general terms it may be stated that:

1. A lymphocytosis indicates a tuberculous process.

2. An abundance of polymorphonuclear and eosinophilic leukocytes indicates a meningococcic, streptococcic, influenza or pneumococcic infection.

When the case is one of meningism there are very few cells. In poliomyelitis there is a cell increase of which 90% may be lymphocytes.

A method of examination considered by neurologists as of differential diagnostic value is to count the number of cells in a cubic millimeter of the cerebrospinal fluid. The technic is to use a gentian-violet-tinged 3% solution of acetic acid. This is drawn up to the mark 0.5, and the cerebrospinal fluid is then sucked up to 11. After mixing, the cell count is made with the hæmocytometer. Normally we have only one or two cells per cubic millimeter, but in tabes or general paresis this is increased to 50 or 100 cells (greatest at onset of disease).

The test for globulins as showing parasyphilitic disease is taken up under Treponema pallidum. The technic of the Wassermann test with cerebrospinal fluid is discussed under that test. Any excess of urea in the cerebrospinal fluid is a sure sign of renal inadequacy.

Trypanosomiasis gives a cellular increase very similar to syphilis.

In the work of the French Sleeping Sickness Commission five cells per cubic millimeter was taken as normal.

CHAPTER XXXIV.

RABIES, VACCINIA AND THE FILTERABLE VIRUSES.

Rabies is a disease of dogs and wolves, but is communicable to man and domesticated animals. The virus, whatever it may be, resides in the saliva and nervous structures. It is destroyed by a temperature of 50° C. In man the period of incubation is usually from three weeks to three months, but may be shorter or may extend over one year.

Bites about the face and those with marked lacerations are particularly serious. Bites of rabid wolves give about four times as great a mortality as those of dogs. In the dog there are two types of the disease—dumb rabies and furious rabies.

By inoculating rabbits subdurally with an emulsion of the brain or spinal cord of a rabid animal, and successively the medulla of this rabbit subdurally into other rabbits, we finally so increase the virulence of the infection that rabbits die in six days. Beyond this it is impossible to increase the virulence and it is termed "fixed virus." The pathogenic power of this virus is also changed so that it is not apt to cause rabies if injected subcutaneously. To attenuate this virus the spinal cord of the rabbit is removed and is dried over caustic potash at a temperature of 23° C. The cord is divided into segments about 1 inch in length. Drying for about fifteen days seems to entirely destroy the virus.

To prepare the material for prophylactic injections a small portion of the cord is emulsified with normal salt solution and injected subcutaneously. The German method is to commence with a cord that has been desiccated only eight days. At first injections are given daily, and it is possible to inject three days' cords by the sixth day. The immunity is "active" and the immunizing agent is a "vaccine." Like vaccine virus the product can be preserved (for probably a month) by the use of glycerine so that it is now possible to send the material for inoculation from the laboratory preparing it.

The treatment lasts for about twenty days. In the diagnosis of rabies in dogs it is preferable to preserve the animal so that the development of the symptoms may be observed.

In case the dog has been killed, it may be possible to make a diagnosis by means of the Negri bodies. These are round or oval bodies from 1 to 20μ in diameter, which may be found in the nerve-cells, especially those of the cornu ammonis (Hippocampus major).

These bodies were first described by Negri in 1903. In street rabies large amœboid forms from 18 to 23μ may be found, while in the nerve tissues of animals with "fixed" virus only minute forms, 0.5μ or less, may be detected. The fact that the

virus will pass through a Berkefeld filter is no argument against its protozoal nature. Calkins considers it to be of rhizopod affinity. The term Neuroryctes hydrophobiæ has been given it. The bodies are present four to seven days before the onset of symptoms. They may be demonstrated by staining smears of gray brain substance by some Romanowsky method, especially by the Giemsa stain. The smears should be made by mashing the thin slice of gray matter taken from 1. Cornu ammonis, 2. Region of fissure of Rolando—in dog crucial sulcus—or 3. Cerebellum, with a cover-glass against the slide. Afterward the cover-glass is gently drawn along the slide.

The smear on the slide is then fixed in methyl alcohol for two to three minutes, washed with water and covered with a stain made by adding 3 drops of Sat. alc. sol. of basic fuchsin to 10 c.c. of distilled water and then adding 2 c.c. of Löffler's methylene blue solution. The stain on the slide is then steamed gently and afterward washed with water and dried.

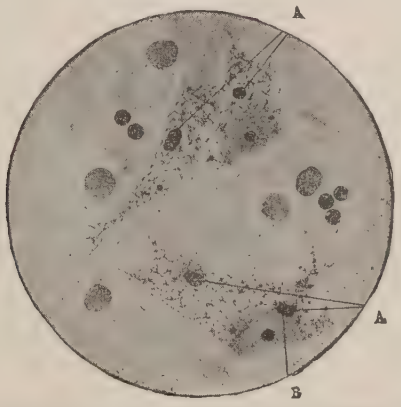


FIG. 105.—Two nerve cells of hippocampus major (smear preparation) showing *Negri bodies*. *A*, *Negri bodies; B*, inner bodies within the *Negri bodies*. (*After Reichel, American Veterinary Review.*)

As their relation to the nerve-cell is more or less disturbed by such a method it is preferable to fix brain tissue from the region of the cornu ammonis for five to seven hours in Zenker's fluid, then to imbed in paraffin and make sections. These are stained with Giemsa's stain and the Negri bodies are brought out as iliac-red bodies in the blue cytoplasm of the nerve-cells. It is necessary to differentiate in 95% alcohol.

In the Lentz method the 3μ sections, after removal of the paraffin, are flooded with absolute alcohol. They are then stained with a 1/2% solution of eosin in 60% alcohol for one minute. Wash in water and next stain for one minute in Löffler's methylene blue. Again wash in water. Apply Lugol's solution to the section for one minute and then differentiate alternately in methyl alcohol and water until the section is pink. After washing in water, again stain with Löffler's blue for one-half a minute, then wash in water and dry carefully with filter-paper. Now differentiate in alkaline alcohol (1 drop of a 5% solution NaOH in 30 c.c. absolute alcohol) until

the section is pink, then quickly differentiate in acid alcohol (1 drop 50% acetic acid in 30 c.c. absolute alcohol) until a slight blue outline to the ganglion cells is obtained. Treat rapidly with absolute alcohol and xylol and mount in balsam. The Negri bodies show as light carmine pink bodies on the light blue ground of the ganglion cells. In the interior of the pink bodies dark blue dots or rings may be observed.

This method can also be used for brain smears.

In addition to examining for the Negri bodies, a rabbit may be inoculated subdurally with a sterile salt-solution emulsion of the medulla of the dead dog.

If the brain and medulla of the dog are to be sent to a laboratory for examination they should be packed in ice or placed in glycerine. Take of glycerine one part and one part water. Sterilize the diluted glycerine by boiling, allow to cool, and drop the pieces of brain tissue into this. This does not kill the virus.

When from advanced putrefaction, or otherwise, the Negri bodies cannot be found the changes in the Gasserian ganglia may give a diagnosis. In typical lesions the ganglion cells are more or less completely destroyed and replaced by cells of other types.

When a person is bitten by a dog suspected of being rabid the following simple measures should be instituted. The dog should be kept under observation in a safe quiet place and will show clinical evidence of rabies within five days and will die shortly afterward in case rabies exists. When the animal dies the head and several inches of the neck should be removed and packed in ice and sent to the nearest laboratory.

Antirabic serum has been prepared by injecting sheep with emulsions of rabbits' cord and brain—at first intravenously, then subcutaneously.

The thorough cauterization of the dog-bite wound with pure nitric acid, as soon as possible after the bite, is imperative even when the Pasteur treatment can be given later.

Vaccinia is a disease produced artifically by the injection of vaccine virus obtained from the calf. The material for vaccine is taken from vesicles about one week after the inoculation. The most potent material is in the pulp at the base of the vesicle and not in the lymph which exudes from the vesicle. The pulp is ground up and mixed with an equal amount of glycerine, which acts not only as a preservative but as a mild antiseptic for nonsporing bacteria. The calves are autopsied after the pulp has been curetted from the inoculated skin of the abdomen to be sure that no disease exists in the calves. The virus is afterward tested for pus organisms, tetanus, and foot and mouth disease.

Guarnieri in 1892 first observed small bodies near the nucleus of infected epithelial cells, He called them Cytoryctes vacciniæ. Calkins regards these bodies as well as the Negri bodies as being rhizopods and the distributed chromatin as idiochromidia (granules of nuclear chromatin within the cytoplasm).

The Filterable Viruses.

The first disease of which the virus was found to be capable of passing through the finest porcelain filter was that of foot and mouth disease (Löffler and Frosch, 1898).

The filter which is ordinarily used for testing for the passage of such disease agents is the Berkefeld filter, one made of diatomaceous earth. Of the infections belonging to man in which such a passage of blood or serum through the pores of a porcelain filter, capable of keeping back even such a small bacterial organism as that of Malta fever, but which does not hold back their virus, we have the following: foot and mouth disease, trachoma, molluscum contagiosum, vaccinia, variola, rabies, typhus fever, measles, scarlet fever, yellow fever, dengue, Papataci fever and poliomyelitis.

There are many diseases of this nature which are important among the domesticated animals, such as pleuropneumonia of cattle, African horse sickness and hog cholera. The viruses of pleuropneumonia of cattle and poliomyelitis have been obtained in artificial cultures. Some of these viruses seem related to bacterial infections and others to protozoal ones. These viruses differ as to method of transmission, pleuropneumonia of cattle being transmitted by inhalation, rabies and vaccinia by the cutaneous atrium, hog cholera by ingestion and many of those supposed to have protozoal affinities, as yellow fever, Papataci fever and horse sickness by mosquitoes.

As a rule these viruses are destroyed by a temperature of 55° C. in a few minutes.

MISCELLANEOUS NOTES.

MISCELLANEOUS NOTES.

APPENDIX.

A—PREPARATION OF TISSUES FOR EXAMINATION IN MICROSCOPIC SECTIONS.

1. **Fixation:**

a. It is most important that the tissues to be examined be placed in the fixing fluid as soon after death or operation as possible. Degenerative changes are in this way avoided.

b. The piece of tissue to be fixed must not be too large. Using a sharp scalpel, or preferably a razor, a slab of tissue about one-half an inch square and not more than one-fifth of an inch thick should be dropped into the bottle containing the fixative. The bottom of this bottle should have a thin layer of cotton with a piece of filter-paper covering it. There should be at least twenty times as great a volume of fixing fluid as of tissue to be fixed. Delicate tissues, as pieces of gut, should be attached to pieces of glass, wood, cardboard, or blotting paper before being placed in the fixative.

c. The most convenient fixative for the average medical man is a 10% solution of ordinary commercial formalin (4% of formic aldehyde gas), either in water or, preferably, in normal salt solution. Fixation is complete in from twelve to twenty-four hours. By placing in the incubator, at 37° C., two to twelve hours in the formalin solution suffices. If fixed in the paraffin oven (56° C.), fixation is accomplished in about one-half hour.

Formalin once used for fixation must be thrown away.

The fixative which probably gives the best histological pictures and with which we obtain the most satisfactory hæmatoxylin staining is Zenker's fluid. This is Müller's fluid containing 5% of corrosive sublimate. It also contains 5% of glacial acetic acid, which latter is only added just before we are ready to fix the piece of tissue. Müller's fluid is:

Pot. bichromate,	2.5 grams.
Sod. sulphate,	1.0 grams.
Water,	100.0 c.c.

Zenker's fluid fixes in about twenty-four hours. After all corrosive sublimate fixatives we should wash the tissues in running water for twelve to twenty-four hours. The precipitate of mercury in the tissues is best gotten rid of by treating the section on the slide with Lugol's solution, rather than the tissue in bulk with iodine alcohol.

In Orth's fluid we add 10% of formalin to Müller's fluid (recommended for nerve tissue).

A saturated corrosive sublimate solution in salt solution with the addition of 5% of glacial acetic acid may be used as a substitute for Zenker's fluid.

2. **Dehydration.**—After washing for twelve to twenty-four hours in running water, following corrosive sublimate fixation, or simply washing for a few minutes after formalin, the tissues should be placed in 70% alcohol. They may be kept in this indefinitely. If they are to be sent to a laboratory for sectioning, it is advisable to moisten a pledget of cotton in 70% alcohol and fill in the bottom of the bottle with it. Then drop in the tissues and pack in gently over them sufficient 70% alcohol-saturated cotton to fill up the bottle. All the alcohol should be absorbed by the cotton so that if the bottle should break in transit there would be no damage from the alcohol. The stopper of the bottle should be paraffined or sealed with wax.

Tissues may be left in the 70% alcohol twelve to twenty-four hours and should then be transferred to 95% alcohol for an equal time. They are then transferred to absolute alcohol, where they remain from two to twelve hours and are then placed in xylol. The time in xylol should be as short as possible. So soon as the tissue looks clear it should be removed—thirty minutes to two hours.

3. **Imbedding.**—The tissue is now transferred to melted paraffin. Paraffin melting at 48° C. for winter work, and that melting at 54° C. for summer is to be recommended. The time in the paraffin should not be prolonged. Two hours will ordinarily suffice. Some leave in the paraffin for twelve to twenty-four hours.

Next take a paper box (made of stiff writing-paper folded over a square of wood) and fill with the melted paraffin. As quickly as possible drop in the piece of tissue taken out of the paraffin bath with heated forceps and, so soon as the paraffin begins to solidify on the surface, place the paper box in ice water. When paraffin is rapidly cooled, crystallization is less.

The Acetone Method.—Take the tissues out of the 70% alcohol and place in acetone. After remaining in acetone for one to two hours, the tissues should be transferred to fresh acetone for an equal length of time. Dry calcium chloride in the bottom of the acetone bottles keeps it dehydrated. They should then be placed in xylol for about one-half hour and then embedded in paraffin as directed above.

The Chloroform Method.—The procedure may be the same as in the method of passing through alcohols to xylol, substituting chloroform for xylol and then transferring to paraffin.

Where absolute alcohol is not obtainable, very satisfactory results may be obtained by transferring to a mixture of 95% alcohol and chloroform after immersion in 95% alcohol. Then going from the alcohol-chloroform mixture to pure chloroform, thence to paraffin.

Rapid paraffin imbedding methods.

When a piece of tissue is not more than one-fourth inch square and one-eighth inch thick, it is very easy to run it through in three to six hours. Thus:

10% Formalin (in 37° C. incubator),	1 hour.
70% Alcohol (in 37° C. incubator),	1 hour.
95% Alcohol (in 37° C. incubator),	1 hour.
Absolute Alcohol (in 37° C. incubator),	1/2 hour.
Xylol (in 37° C. incubator),	1/2 hour.
Paraffin (in 55° C. incubator),	1/2 to 2 hours.

Method of Lubarsch.—In this excellent method small pieces of tissue not more than 1/5 inch thick are placed in a wide test-tube containing 10% formalin for 10 to 15 minutes, changing the fluid twice. Transfer to 95% alcohol 10 minutes changing alcohol once. Absolute alcohol, for 10 minutes changing twice. Pure aniline oil until tissues are transparent, 15 to 30 minutes. Xylol, changing two to three times or until the xylol is no longer yellow, 10 to 20 minutes. Imbed in paraffin for 20 minutes to 1 hour. During the entire process keep the test-tube in a water bath or incubator at 50° C. It is preferable to have a good microtome. The best is that of Minot. Very satisfactory sections can be cut with the various types of student microtomes, costing from $12 to $20.

(In using a hand microtome, a razor with a flat edge is necessary. After experience, sections thin enough for histological but not for bacteriological examination can be made.)

If the piece of tissue is properly dehydrated and imbedded, thin sections (3 to 10μ) should be easily obtained, provided the knife be sharp. One advantage about the paraffin method is that it is only necessary to have a small part of the blade in proper condition. With celloidin the entire cutting edge must be perfect. Having cut the sections, they should be dropped on the surface of a bowl of warm water (45° C.). This causes the section to flatten out evenly.

Decalcification.—This is best accomplished by fixing in 10% formalin for twenty-four hours, then placing a small piece of the bone (not exceeding one-half inch square and one-fifth of an inch thick) in concentrated sulphurous acid.

This decalcifies in about 2 to 7 days. Wash thoroughly in alkaline water and then in tap water. Pass through alcohols and xylol and imbed and section as before described.

To Stain Sections.—It is first necessary to affix the section to a slide or cover-glass.

To attach the section firmly to the slide, so that it will not become detached in subsequent treatment, pick up a section on a strip of cigarette paper.

A sheet of cigarette paper is cut into about five pieces (1/2×1 1/2 inches). Inserting the strip of cigarette paper under the section, it is easily lifted up out of the water. Then apply the slip of cigarette paper, section downward, to a perfectly clean slide. Blot with a piece of filter-paper, then strip off the piece of filter-paper leaving the section smoothly applied to the slide. Next place in the 37° C. incubator for twelve to twenty-four hours and the section will be found to be so firmly attached that it will not be dislodged by subsequent treatment.

For Immediate Diagnosis.—Take a loopful of albumin fixative (white of fresh egg, 50 c.c.; glycerin, 50 c.c.; sodium salicylate, 1 gram) and deposit it on a cover-glass. Now take up a loopful of 30% alcohol (1 drop of 95% alcohol and two drops of water) and applying it over the albumin fixative, smear out the mixture uniformly over the cover-glass.

2. Pick up a section on a strip of cigarette paper and apply it to the prepared surface on the cover-glass. Blot with gentle pressure with a piece of filter-paper over the strip of cigarette paper, and strip off this latter, leaving the section attached to the cover-glass.

3. Now, turning the flame of the Bunsen burner down very low or with a small alcohol flame, we hold the cover-glass in a Stewart's forceps, section side up, over the flame and slowly lower it until the paraffin is observed to melt. This shows a

temperature of about 50° C. The section is fixed by the coagulation of the albumin at about 70° C. To obtain this temperature lower the cover-glass still more, and the moment vapor is seen to rise from the section it indicates the attachment of the section to the cover-glass.

4. Flood section on cover-glass or slide with xylol; this dissolves out the paraffin. It is better to pour off the first xylol and drop on fresh xylol (one minute).

5. Remove xylol with two applications of absolute alcohol (one minute).

6. Treat specimen with two or three applications of 95% alcohol (one to two minutes).

7. Next wash in water (one to two minutes).

8. Flood specimen with hæmalum or Delafield's hæmatoxylin (three to seven minutes).

9. Wash in tap water for about two to five minutes until a purplish tinge is developed in the section. The alkali in ordinary tap water develops this color.

10. Apply 1 to 1000 eosin for thirty seconds to one minute.

11. Wash in water; then in 95% alcohol; then in absolute alcohol.

12. Apply a few drops of xylol and as soon as the section is perfectly transparent mount in balsam.

The staining by hæmatoxylin and eosin is the best for the study of the histology of a section. It only requires about ten minutes to run a preparation through for diagnosis by this method.

The reagents are best kept in dropping-bottles.

The staining of sections on slides is exactly as for those on cover-glasses. Coplin's staining jars are very convenient for use in staining slides.

Where the cover-glass method is used, staining by Gram's method, acid-fast staining, capsule staining, etc., may be carried out as for bacterial preparations.

For staining Gram positive bacteria in sections, the Gram method as for bacterial preparations, using dilute carbol fuchsin as a counterstain, gives good results.

For Gram negative bacteria stain with thionin as for blood preparations (ten to twenty minutes). Then differentiate in 1 to 500 acetic acid solution for ten to twenty seconds, wash with water, then with 95% alcohol, and quickly through absolute alcohol and xylol.

Nicolle's Method.—1. Stain with Löffler's methylene blue ten to fifteen minutes.

2. Differentiate in 1 to 500 acetic acid ten to twenty seconds.

3. Place in 1% solution of tannin for a few seconds (fixes color).

4. Wash in water, then into 95% alcohol, absolute alcohol, xylol, and balsam.

Weigert's Iron Hæmatoxylin.

Solution I.

Hæmatoxylin,	1 gram.
Alcohol (95%),	100 c.c.

This must be allowed to ripen for some days and does not keep over six months.

Solution II.

Liq. Ferri sesquichlor. sp. gr. 1.124 (about 10%)	4 c.c.
HCl,	1 c.c.
Water,	100 c.c.

Mix equal parts of number one and number two. The mixture only keeps about three days. The HCl prevents overstaining.

This stain followed by Van Giesen's stain gives more perfect results than any common method of staining. The iron hæmatoxylin intensifies the sharpness of the Van Geisen differentiation.

Van Giesen's Stain.—Take of 1% aqueous solution acid fuchsin from 5 to 15 c.c. Saturated aqueous solution picric acid 100 c.c. The method of using is to first stain with hæmatoxylin in the usual way. Then pour on the picric-acid fuchsin solution and allow to stain for one to five minutes. Wash, pass through alcohols and xylol and mount in balsam.

Connective-tissue fibers, axis cylinders, and ganglion cells are stained a bright garnet red. Myelin, muscle fibers, and cells generally are stained yellow. Nuclear staining is that of hæmatoxylin. The stronger stain is used for nerve tissue; the weaker, for demonstrating connective tissue in tumors.

Levaditi's Method.—Take small pieces of tissue, about 2 mm. in thickness, and harden in 10% formalin for twenty-four hours and then in alcohol for the same period; then wash in water for a short period. They are stained in a freshly made solution of silver nitrate 1.5% for three successive days, changing the solution each day, maintaining the blood temperature, and excluding light. The tissue is then placed in a 2% solution of pyrogallic acid, with the addition of 5% formalin. After remaining in this for twenty-four hours, light being excluded, they are passed through 85%, 95%, and absolute alcohol, respectively; embedded in paraffin; and cut in about 5 micron sections. Equally good results may be obtained by allowing the silver nitrate to act at room temperature and embedding in celloidin.

Romanowsky.—Staining sections with Romanowsky stains is not very satisfactory. The differential staining seems to fade out in passing through the alcohols. This may be avoided by blotting the section after staining and differentiation and then applying the xylol to the blotted section. After staining with Giemsa's stain for ten to fifteen minutes, differentiate with 1 to 500 acetic acid. When the section has a pinkish tinge, wash in water, dry, clear in xylol, and mount.

Good tissue staining may be gotten with Wright's stain. After removing the paraffin with xylol and the xylol with absolute alcohol, pour on a sufficient number of drops of stain and immediately dilute with an equal number of drops of water. Allow the diluted stain to remain for three to five minutes. Next wash in water, differentiate, until the tissue has a pinkish tinge, in 1 to 500 acetic acid. This differentiation is best done in a tumbler of the dilute acetic acid.

After washing in water, quickly pass through 95% and absolute alcohol, clear in xylol, and mount.

Skin Sectioning.—Of all tissues that of skin offers the greatest difficulty in preparing sections. The best results can probably be obtained by fixation in picro-sublimate (saturated aqueous solution picric acid 1 part; saturated aqueous solution bichloride of mercury one part); to this stock mixture add 5% glacial acetic acid just before using. Fix small pieces of skin six to eighteen hours. Transfer direct to 70% alcohol in which the tissue may be kept indefinitely.

For sectioning run through alcohols to absolute and then to a mixture of absolute alcohol and carbon bisulphide (equal parts). Leave until tissue sinks, then transfer to pure carbon bisulphide until tissue sinks. Then transfer to a saturated

solution of paraffin in carbon bisulphide and thence to paraffin. Bisulphide of carbon has the disadvantage of foul odor and inflammability but does not seem to render tissues brittle and difficult to section as does xylol.

Neurological Staining Methods.

Neuropathology practically dates from the introduction of Marchi's method of staining in 1885.

Ordinary osmic acid stains both normal and pathological fat. With Marchi's method only the oleic acid of fatty degeneration is stained.

The method is not useful until three or four days have elapsed from the onset of the condition causing the degeneration and it is applicable for only three or four months because by that time phagocytes have taken up the pathological fat which is stained in the Marchi method. The Weigert method is the one to use after a period of three or four months. In Weigert's stain only the normal myelin sheath is stained and the lack of staining of myelin sheaths in degenerated areas is the basis of the stain. For demonstrating axonal reactions or other degenerative changes in nerve cells, as shown by bulging of the concave sides of the cells, eccentric nucleus and granular appearance of the tigroid bodies, Nissl's method is the best.

For neuroglia fiber staining Mallory's phosphotungstic acid hæmatoxylin is to be recommended.

I. **For Marchi's Method.**—Small pieces of nerve tissue are hardened in Müller's fluid for seven to ten days and are then transferred to a mixture of two parts Müller's fluid and one part of a 1% osmic acid solution and should remain in this mixture for about seven days. The tissue thus treated is run through alcohols and imbedded in paraffin in the usual way.

II. **For Weigert-Pal Method.**—Thin slices of tissue are fixed in 10% formalin in about four days. The tissue should then be transferred to 5% potassium bichromate for about twelve days. The tissue is then imbedded and sections cut. If only recently mordanted these sections may be at once stained with Weigert's hæmatoxylin for twelve to twenty-four hours (10 c.c. ripened 10% solution hæmatoxylin in absolute alcohol and 90 c.c. water). Wash in water to which about 2% of a saturated solution of lithium carbonate has been added. Now differentiate from one-half to five minutes in 1/4% solution of potassium permanganate until the gray matter looks a brownish-yellow. Next treat sections with oxalic acid 1 gram, potassium sulphate 1 gram and water 200 c.c. until the gray matter is almost colorless. This takes only a few seconds. Wash in water, pass through alcohols and xylol and mount in balsam.

III. **For Nissl staining** either thionin or Giemsa staining is satisfactory.

IV. For neuroglia fiber staining use **Mallory's Phosphotungstic acid hæmatoxylin.**

Take of hæmatein ammonium,	0.1 gram.
Water,	100.0 c.c.
Phosphotungstic acid crystals (Merck)	2.0 grams.

Dissolve the hæmatein in a little water with the aid of heat, and add it after it is cool to the rest of the solution; no preservative is required. If the solution stains weakly at first, it may be ripened by the addition of 5 c.c. of a 1/4% aqueous

solution of potassium permanganate, or it may be allowed to stand for a few weeks until it ripens spontaneously.

Making and Staining of Frozen Sections.

The various types of ether freezing microtomes are not very satisfactory when only used occasionally. With the general introduction of cylinders containing compressed carbon dioxide, which is used for aerating waters, we have at hand a practical and convenient method of making frozen sections.

The instrument makers furnish a freezing microtome of the Bardeen type which can be attached directly to the cylinder by a revolving clamp nut.

It is necessary to have a stand to support the iron cylinder in a horizontal position The tissue, which may be taken at operation for immediate diagnosis, or which preferably has been fixed in formalin for twelve to eighteen hours is immediately placed in water. If the tissues have been in alcohol it will require hours of washing before they can be frozen. The piece of tissue which is to be frozen should not be more than one-fifth of an inch thick. Having placed the piece of tissue on the freezing box of the microtome we turn the valve of the cylinder to allow the gradual escape of gas. When frozen solid, we elevate the freezing box holding the frozen tissue, by revolving a graduated disc with the left hand. In the right hand we firmly grasp a well-sharpened blade of a carpenter's plane mounted in a wooden handle. This is held at an angle of 45 degrees to the polished ways of the microtome. By alternate shoving and withdrawing of the blade, held rigidly, we accumulate on the blade a number of sections. Then dip the blade in a vessel of water to detach the sections which float in the water. Keep repeating the process until numerous satisfactory sections are obtained. Handles for holding the Gillette razor blades are good substitutes for the carpenter's plane.

These sections may be picked up with a strip of cigarette paper and applied to a clean slide upon which a very small loopful of albumin fixative has been smeared out with 30% alcohol. The piece of cigarette paper with the section underneath is then firmly smoothed out upon the slide with filter-paper. The piece of cigarette paper is then carefully stripped off and the section remains attached to the slide. By careful heating over a very small flame, until the vapor just arises, the section is fixed to the slide and we can then stain the section in any way that may be desired.

Note.—The procedures for carrying the tissues through celloidin are not given as it requires perfect condition of the entire cutting surface of the microtome knife and a considerable time for the passage through regents and celloidin. It is more suitable as a method when sections for class work are to be prepared.

B—MOUNTING AND PRESERVATION OF PATHOLOGICAL SPECIMENS AND ANIMAL PARASITES.

To Mount Small Round Worms.—Wash the hook, whip, or filarial worm in salt solution, then drop in 70% alcohol containing 5% of glycerine; the glycerine-alcohol mixture being at a temperature of 60° C. When cool, pour into Petri dishes and allow the alcohol to evaporate in the 37° C. incubator.

Mount in glycerine jelly, preferably in a concave slide, and ring the preparation

with gold size. The following is the formula for Kaiser's glycerine jelly: Soak one part of gelatin in six parts of distilled water for two hours. Then add seven parts of glycerine. To the mixture add 1 % of carbolic acid, warm for fifteen minutes, with constant stirring, and then filter through cotton.

To Prepare Tape-worms.—Wash in salt solution. Wrap around a piece of glass as a glass slide and fix in salt solution containing 2 to 5% of formalin. Then keep the preparation permanently in 70% alcohol. If preferred, the specimen may be run through alcohols and xylol and mounted in balsam.

Larvæ.—Mosquito larvæ may either be prepared as for small round worms or they may be dropped into 70% alcohol at 60° C. and then passed through alcohols and cleared in xylol and mounted in balsam. Flukes and insects may require treatment with hot (60° to 70° C.) solution of 10 to 20% sodium hydrate solution. Then wash thoroughly in water and subsequently pass through alcohols to xylol and mount in balsam. Clove oil or cedar oil clears more slowly, but makes specimens less brittle than does xylol. Another satisfactory method is to drop insects or larvæ into acetone at 60° C. and after being in this from one to twelve hours to clear in xylol or clove oil and mount in balsam.

Nematodes.—Looss has a method of first washing a small nematode or delicate fluke in salt solution. Then pouring this first salt solution out of the test-tube in which the washing was carried out, to add fresh salt solution, and then an equal amount of saturated aqueous solution of bichloride of mercury. The shaking is easily carried on in the test-tube. After washing in water the worm is passed through alcohols, one strength of which should contain iodine. Clear in xylol and mount in balsam.

An excellent method is that of Langeron.

After washing in salt solution fix for a few hours in 5% formalin. Then transfer to lactophenol which has been diluted with an equal amount of water. Allow to remain in this solution for several hours and then transfer to pure lactophenol in which fluid the specimens are to be mounted. Ring with paraffin or with gold size. (To make lactophenol take two parts of glycerine and one part each of distilled water, crystallized carbolic acid and lactic acid.)

A quick method of preparing small nematodes for examination is to fix them for from two to twelve hours in 5 to 10% formalin, this being heated to 60° C. at the time the worms are dropped into it. Then transfer to the following solution:

Glucose syrup (glucose, 48; water, 52),	100 c.c.
Methyl alcohol,	20 c.c.
Glycerine,	10 c.c.
Camphor, q.s. (a small lump for preservation).	

They may be mounted directly in this and the cover-slip ringed with about 60° C. paraffin, followed with gold size.

Preparations so cleared and mounted in glycerine jelly should also be ringed with paraffin or some cement.

Flukes, cestodes, and nematodes are best stained with carmine. The following is a good formula.

Dissolve, by boiling, 4 grm. carmine in thirty drops HCl and 15 c.c. water. Then

add 95 c.c. of 85% alcohol and filter while hot. Neutralize with ammonia until precipitate begins to form. Then filter cold.

1. Stain parasites taken from 70% alcohol for five to twenty minutes. 2. Differentiate in 3% hydrochloric acid. 3. Pass through alcohols to xylol and mount in balsam.

Mites, Fleas and Various Small Insects.—By simply taking one or two drops of liquid petrolatum and mounting the specimen in it then covering with a coverglass one is able to study the details of these objects almost as well as if they were passed through acetone and xylol into balsam. Liquid petrolatum is also most excellent for mounting the aërial hyphæ of fungi with their sporangia as well as for Romanowsky stained blood smears.

Pathological tissues which are to be sent to a laboratory for sectioning or to be kept for future study should be fixed by one of the methods given in Section A of the appendix.

Formalin fixation is the more convenient—that with Zenker's fluid the more perfect. After fixation with Zenker's fluid the pieces of tissue must be washed in running water over night.

After fixation the pieces of tissue are transferred to 70% alcohol in which they may be kept indefinitely.

For preservation of gross specimens the method KAISERLING is generally used.

Fix for from one to five days in Solution I.

Solution I.

Formaldehyde,	200 c.c.
Water,	1000 c.c.
Nitrate of potassium,	15 grams.
Acetate of potassium,	30 grams.

The position of the specimen should be changed from day to day. There must be at least five times as much fluid as specimen. Drain and transfer to 80% alcohol for a few hours, then into 95% alcohol until the natural color is just restored.

Finally preserve in

Acetate of potassium,	200 grams.
Glycerine,	400 c.c.
Water,	2000 c.c.

It is advisable to keep these specimens in the dark as light destroys the natural color.

To Prepare Flies or Mosquitoes for Transmission Through the Mails.—Wrap the insect carefully in a piece of tissue paper (toilet-paper answers). Impregnate sawdust with 5% carbolic acid solution and fill around the tissue paper in the box containing them. (Barely moisten.)

It is very satisfactory to take a tube form vial with a cork from the inner surface of which two small shallow holes have been bored, one containing paraformaldehyd, the other camphor. The insect is mounted upon a pin stuck in the cork, which latter is inserted and parafined externally.

C—PREPARATION OF NORMAL SOLUTIONS.

A normal solution is one which contains the hydrogen equivalent of an element, expressed in grams, dissolved in sufficient distilled water to make 1000 c.c. The hydrogen equivalent is the atomic weight of any element divided by its valence. In a base, salt or acid, we use the molecular weight in grams divided by valence.

What may be considered as the valence of a base is shown by the number of hydroxyls combined with it; that of an acid by the number of replaceable hydrogen atoms which it contains.

To make a normal solution, dissolve in distilled water a weight in grams equal to the sum of the atomic weights of the substance divided by its valence, and make up the volume to exactly 1000 c.c.

NaOH is univalent. Na = 23. O = 16. H = 1. Dissolve 40 grams NaOH in water and make up to exactly 1000 c.c.

Oxalic acid is COOH—COOH + $2H_2O$ which gives it a molecular weight of 126. As it contains two carboxyl groups it is dibasic, and it is necessary to divide the molecular weight by 2, so that for a normal solution of oxalic acid we dissolve 63 grams in a volume of distilled water made up to 1000 c.c.

If a chemical laboratory is not accessible one may prepare normal solutions with an error so slight as to be unimportant in clinical work in the following way:

Sodium hydrate being very hygroscopic, it is impossible to accurately prepare a normal solution by directly weighing out the substance. Instead, select perfect crystals of oxalic acid, such as can be obtained in a drug store, and weigh out on the most accurate apothecary scales obtainable exactly 6.3 grams of the most perfect crystals in the bottle. Put these preferably in a volumetric flask and make up with distilled water to 1000 c.c. Less accurate is the use of a measuring cylinder. If care is used this should give N/10 solution of oxalic acid in which the error is less than 1%.

Having N/10 acid at hand, we may prepare N/10 NaOH in the following way: Weigh out an excess of sodium hydrate (5 grams of stick caustic soda) and dissolve in 1100 c.c. of distilled water. Take up 10 c.c. of this solution with a pipette and let it run into a beaker. Add six drops of phenolphthalein solution. This gives a violet-pink color. Fill the burette with the N/10 oxalic acid solution and let it run into the sodium hydrate solution in the beaker until the pink is just discharged. Reading off the number of c.c. of the N/10 acid used, we know the strength of the sodium hydrate solution. It is well to repeat the titration and take an average.

If 10.5 c.c. of the oxalic acid solution were required it would show that the sodium hydrate solution was stronger than N/10, as only 10 c.c. would have been necessary if the NaOH solution had been N/10. It is therefore necessary to dilute the sodium-hydrate solution in the proportion of 10 to 10.5. Measure exactly 1000 c.c. of the too concentrated sodium-hydrate solution and add to it 50 c.c. of distilled water, mix thoroughly, and we have 1050 c.c. of N/10 solution of NaOH. $1000 \times 10.5 = 10{,}500$. $10{,}500 \div 10 = 1050$.

As Acidum hydrochloricum U. S. P. is about two-thirds water (68.1%) to make N/10 HCl, which would require 3.65 in 1000 c.c., it would be necessary to take about three times this amount of U. S. P. acid. Take 12 c.c. of the acid and add distilled water to make 1100 c.c. Put 10 c.c. of this dilute solution in a beaker. Add phenol-

phthalein solution and titrate. If 11 c.c. of N/10 NaOH were required it would be necessary to add 100 c.c. of water to a volume of 1000 c.c. of the diluted hydrochloric acid. $1000 \times 11 = 11000 \div 10 = 1100$.

Other acid and alkali solutions can be made as for N/10 HCl and N/10 NaOH.

D—DISEASES OF UNKNOWN OR NOT DEFINITELY DETERMINED ETIOLOGY.

Of Temperate Climates.

Acute Articular Rheumatism.—Various bacteria have been reported as cause.

Epidemic Poliomyelitis.—Material from the cord of child with the disease when injected subdurally, intravascularly, or into the peritoneal cavity of monkeys produced the disease in the animals inoculated. The virus has been passed through three generations of monkeys (Flexner).

The virus has been found in the brain, spinal cord, mesenteric and salivary glands of monkeys and may remain in the nasal muscosa of monkeys as long as five months. This would indicate the existence of human chronic carriers. With the possible exception of the rabbit only man and the monkey are susceptible. This would indicate that the virus is directly transferred from man to man. The virus is highly resistant to drying and light. It will remain alive for months in dust. It is not sterilized by pure glycerine during many months of contact. It is possibly transmitted by a biting fly, Stomoxys calcitrans.

Flexner and Noguchi have recently cultivated the virus of poliomyelitis by employing ascitic fluid to which had been added a fragment of sterile rabbit kidney and nutrient agar, this culture medium being covered with a layer of paraffin oil. The growth is obtained under anaerobic conditions. The minute colonies are composed of globular or globoid bodies from .15 to .3 mikron in diameter. These bodies may be single or in chains or in masses. In older cultures bizarre forms are obtained. Monkeys have been inoculated with the cultures.

Foot-and-mouth Disease.—Probably due to an ultramicroscopic organism.

Measles.—Cause entirely unknown. Hektoen has shown that blood contains the virus.

Anderson has found that the virus of measles can pass through a Berkefeld filter and loses its infectivity after heating for 15 minutes at 55° C. In infecting monkeys it was found that the blood of patients with measles was infective only just before and for about twenty-four hours after the appearance of the eruption. Mixed nasal and buccal secretions were infective for monkeys for about forty-eight hours from the time of the eruption. The scales from desquamating cases were not capable of infecting monkeys hence it was thought that measles was not contagious during the period of desquamation.

Mumps.—Herb has implicated a diplococcus. Inoculations into Steno's duct of monkeys successful.

Rabies.—Probably the Negri bodies.

Roetheln (German Measles).— Nothing known.

Scarlet Fever.—Streptococci seem most probable cause (S. anginosus). Mallory has implicated epithelial protozoa.

Recently cell inclusions in polymorphonuclears have been supposed to be diagnostic; other diseases however seem to give them as measles, diphtheria, etc.

Small-pox and Vaccinia.—Guarnieri and Councilman have implicated epithelial protozoa.

Spotted Fever of the Rocky Mountains.—Supposed to be due to an unknown protozoon transmitted by a tick, D. andersoni.

Typhus Fever.—It has been suggested that the cause may be a protozoon transmitted by vermin.

Recent work by Anderson and Ricketts has shown that the blood of human cases is infective for monkeys. The virus does not seem to pass through a Berkefeld filter and the epidemiology points to the body louse as the transmitting agent.

Varicella.—Entirely unknown.

Whooping Cough.—Influenza-like bacilli have been implicated. Bordet-Gengou bacillus.

Of Tropical Climates.

Ainhum.—A disease characterized by a constricting fibrous ring, especially of little toe, often leading to spontaneous amputation.

Beriberi.—Various microorganisms and food factors suggested. A form of multiple neuritis, occurring chiefly in countries where rice is the staple food, characterized by œdema and marked cardiac and respiratory embarrassment. The vagal involvement produces grave symptoms. Rice from which the pericarp has been largely removed, polished rice, implicated.

Blackwater Fever.—Considered as a malarial disease, but thought by some to be possibly caused by a protozoon—a Babesia (Piroplasma). A disease usually occurring in malarial patients characterized by rapid febrile onset, early jaundice, asthenia, pain in loins and the pathognomonic hæmoglobinuria.

Dengue.—Supposed to be due to a protozoon transmitted by Culex fatigans. A disease characterized by sudden onset, high fever for three or four days, pains in the postorbital regions, back and about joints. A remission occurs on the third to fifth day followed by a secondary rise of temperature and a measles-like eruption. Leukopenia and reduction in the percentage of polymorphonuclears. Virus exists in the blood and is filterable.

Goundou.—Symmetrical bony tumors of nasal processes of superior maxillary bones.

Pellagra.—A disease about which two etiological views exist (1) that it is connected with the ingestion of spoiled maize, the other that it is of protozoal nature and transmitted either by Simulium reptans or Stomoxys calcitrans. It is characterized by (1) a sprue-like stomatitis and disorders of alimentary canal (2) an erythema usually limited to parts exposed to the sun and characterized by marked symmetry and striking delimitation from the sound skin and (3) various neurological manifestations and a toxic psychosis which may go on to confusional insanity. The disease is characterized by annual recurrences in the spring with improvement in the winter.

Rat-bite Disease.—A disease caused by the bite of rats. Rather common in Japan. Five weeks after bite when wound has healed, high fever sets in, cicatrix

becomes inflamed with lymphangitis and swollen glands. The fever falls in a few days to be succeeded by other febrile paroxysms. An erythematous eruption accompanies the second paroxysm. Supposed to be due to a protozoon.

Sprue.—A form of chronic diarrhœa characterized by diaphanous thinning of gut and ulcerations of buccal cavity.

Tsutsugamushi.—A disease of Japan somewhat resembling typhus fever. Supposed to be due to a protozoon transmitted by the Kedani mite.

Verruga Peruana.—A disease with a fever characterized by a profound involvement of the bone marrow producing very rapidly an anæmia resembling that of pernicious anæmia. Pains of bones and joints marked. Death may occur before the eruption, which appears as patients improve, either as red spots which enlarge to the size of pea (miliary) or as pedunculated lesions as large as a pigeon's egg (nodular). The lesions are very hæmorrhagic and may appear in crops. The nodular form is located chiefly about the knees and elbows but the miliary form may cover entire skin surface and mucous membranes.

Yellow Fever.—Supposed to be due to a protozoon transmitted by the Stegomyia calopus. A disease characterized by sudden onset, rachialgia, albuminuria and jaundice about the third day. Pulse becomes slow even with rising temperature. Black vomit often precedes fatal termination. Virus exists in the blood and is filterable.

E—CHEMICAL EXAMINATION OF URINE.

For the prevention of decomposition when a urine is not examined shortly after voiding, chloroform (10 to 20 drops added to a tightly corked bottle) or formalin (4 or 5 drops to a pint of urine) are ordinarily employed. Formalin is better for microscopical material, but, owing to its reducing power, should be substituted by boric acid in urine to be examined for sugar. For clearing urine, turbid by reason of bacteria, rubbing up with Talcum purificat. U. S. P. and filtering is recommended.

A twenty-four-hour specimen is necessary for accurate work. The urine should be collected in clean separate bottles. Where pus comes from the bladder the proportion of pus in each bottle will be practically the same; if from the kidneys the amount will vary in the different bottles.

The amount of urine varies in different individuals (water or beer habit). It is usually given as from 1000 to 1500 c.c.

Long proposes to substitute 2.6 for Hæser's coefficient which, if multiplied by the two final figures of the specific gravity taken at 25° C., gives the weight of urinary solids in 1000 c.c.

Albumin.—Practically serum albumin alone is clinically important.

The two usual tests are 1. Heat test and 2. Heller's nitric acid test. For the former, add 3 to 10 drops of 5% acetic acid to the perfectly clear urine in a test-tube and bring to a boil. By boiling the upper portion a turbidity in contrast with the clear lower portion may be obtained.

A more delicate test for albumin is the following: Add to a test-tube half filled with filtered urine one-fifth it's volume of a saturated aqueous solution of sodium chloride; heat to the boiling point; add two to five drops of fifty per cent. acetic acid and heat again. This test may serve to distinguish nucleo-albumin, as most

forms of nucleo-proteid found in urine do not react to the test, while serum albumin does. Thus where a positive nitric acid test is present, and no precipitate occurs with this test, the proteid present is usually nucleo-proteid.

For Heller's test, pour a small amount of nitric acid into a narrow test-tube and, while holding the tube at an angle of about 45°, superimpose a layer of the urine to be tested, which is delivered drop by drop from a pipette and allowed to flow down the side of the tube.

This test can be converted into a quantitative one which is sufficiently accurate for clinical purposes. It is based on the fact that a specimen of urine containing 0.003% of albumin will give a perceptible ring at the layering of the urine and acid in two minutes. If the ring appears at once or in a few seconds the albumin content is greater. From the qualitative test an idea can be formed as to the amount of albumin which the urine contains, a heavy ring forming immediately showing a considerable albumin content. Probably the highest elimination of albumin is found in chronic parenchymatous nephritis where it may run from 1 to 3%. In an ordinary case of acute nephritis 0.5% would be an average content.

Recently I have been using for both qualitative and quantitative albumin tests the apparatus shown in Fig. 7. This is simply a five-inch piece of one-fourth-inch soft glass tubing heated at a point 2 inches from one end, drawn out for about two inches and bent to form a U tube with one end shorter than the other. This form of tube enables one to perform two tests with the same column of nitric acid and is easily cleaned and dried. They may be kept suspended around a glass tumbler's rim. Taking up a small amount of nitric acid with a capillary bulb pipette it is deposited in the capillary curve of the bent tube. This acid pipette should be kept attached to the acid bottle. With a second pipette the urine is deposited in the short arm of the U tube and the presence of albumin shows by a distinct ring at the junction of urine and acid in the clear capillary tubing. The long arm will serve for the introduction of a second specimen of urine for the albumin test.

For quantitative test we dilute the filtered urine with one or more parts of normal salt solution according to the intensity of the albumin ring. A very convenient way of making the dilution is with a graduated centrifuge tube. Make a one to ten dilution of the urine, mix and draw up with a bulb pipette and deposit in the short arm of the U tube. A distinct ring forms in 2 or 3 seconds. Pour off one-half of the diluted urine and make up with an equal amount of saline. Deposit this 1–20 dilution in the long arm. The ring forms in about a minute. With further testing it is found that a one to forty dilution shows a perceptible ring in just 2 minutes. This final and successful dilution multiplied by 0.0033 gives the percentage of albumin in the urine ($40 \times 0.0033 = 0.13\%$).

Should it be desired to determine the nature of the proteids present either in urine or in exudates or transudates the following method is applicable. Determine the percentage of total proteid by the method employed above. Then throw down the globulins by the addition of an equal amount of a saturated solution of ammonium sulphate, filter and estimate the proteid content of the filtrate. The difference between that and the total gives the percentage of globulin. The filtrate is now treated with 5% acetic acid until a precipitate of nucleo-proteid ceases to form; the fluid is filtered and the clear filtrate (which should not show any turbidity with a drop of 5 % acetic acid) is tested for its proteid content, which represents the

serum albumin. When the combined percentage of globulins and serum albumin is subtracted from the total proteid percentage we have the percentage of nucleo-proteid.

Bence-Jones Body.—(Albumose.) Perform the heat test for albumin. The appearance of a heavy precipitate which partially clears on boiling suggests albumose. If albumose is present a cloud will appear in the filtrate on cooling. The precipitate formed with nitric acid, if due to albumose, disappears with heat, that of serum albumin does not.

As another test for the *Bence-Jones body*, usually present in multiple myelomata, that of Boston is of value. Mix 15 c.c. urine in a test-tube with an equal amount of saturated NaCl solution. Add 2 c.c. of 40% NaOH solution and shake the contents of the tube thoroughly. Heat the upper contents of the tube to boiling and add lead acetate solution (10%) drop by drop continuing the heating. A brown to black precipitate (sulphur) shows this form of albumin.

In tests requiring the removal of albumin boil the urine and add dilute acetic acid until the precipitate is flocculent, then filter.

Sugar.

Fehling.—Pour equal parts of Fehling's copper solution (34.639 grams of copper sulphate in 500 c.c. of water) and Fehling's alkali solution (173 grams sodium potassium tartrate and 50 grams sodium hydrate in 500 c.c. water) into a test-tube. Mix and dilute the deep blue solution with two parts of water. Heat the upper portion of the diluted Fehling's solution in the flame to boiling and drop in from a pipette the urine to be examined. A yellowish to red precipitate shows the presence of sugar.

Fehling's test will show the presence of 1/100 of 1 % of glucose in an aqueous solution but is vastly less delicate for sugar in urine. This is due to the power of the creatinin in urine of holding the reduced suboxide of copper in solution. An important point is that the creatinin is broken up by prolonged boiling hence the puzzling precipitates one gets at times after a long period of boiling are explained in this way. Glycuronic acid may cause a doubtful reaction. If the precipitated cuprous oxide is in very fine granules the color is greenish, if less fine, greenish-yellow and if quite coarse, reddish.

Creatinin holds in solution the copper suboxide formed by uric acid as well as that resulting from very small glucose content of urine.

As a test for doubtful glycosuria it is well to give 100 grams of pure glucose. A normal person should deal with such an amount without showing sugar reaction of the urine.

Phenylhydrazin (Kowarsky).—Mix five drops of pure phenylhydrazin in a test-tube with ten drops of glacial acetic acid. Shake lightly and add 15 drops of saturated solution of NaCl. This makes a pasty mixture. Now add 10 c.c. of the urine and bring carefully to a boil over a small flame and continue to boil gently for two minutes. Upon cooling a yellowish crystalline precipitate falls more or less rapidly according to the sugar content of the urine. If the urine contains 0.2% or more of sugar the precipitate appears in a few minutes. The test is sensitive for 0.03% of sugar.

Fermentation Test.—This is the surest test for sugar in the urine. It will show the presence of 0.05% of glucose. Instead of the Einhorn apparatus one may be extemporized by taking a 50 c.c. cylinder, filling it to overflowing with the urine which has previously been rubbed up with a piece of compressed yeast the size of a hazel-nut. The urine should be made acid with tartaric acid to prevent ammoniacal decomposition with the formation of CO_2. A small 3-in. test-tube is filled with the yeast-treated urine and dropped mouth downward into the 50 c.c. cylinder. The apparatus is incubated for twenty-four hours and the presence of gas in the closed end of the test-tube shows that sugar was present. A control to determine that the yeast does not contain sugar is advisable. To utilize this test as a quantitive one, first accurately take the specific gravity of the urine; then add the yeast and fill the test-tube and cylinder as directed above. Next pour off or pipette off the urine exactly to the 50 c.c. mark. Incubate for twenty-four to forty-eight hours and make up the loss by evaporation, with distilled water. After the urine has cooled down to room temperature the contents of tube and cylinder are thoroughly mixed (the small tube having been withdrawn with a pair of forceps), then filtered to remove the sediment of yeast and then brought to the exact original volume of 50 c.c. with distilled water to make up the loss by evaporation. (If there should be doubt as to the completion of the fermentation of the glucose a qualitative test for sugar can be made.) The specific gravity is again taken and the difference between this and the first reading multiplied by 0.23. Example: Specific gravity of unfermented urine, 1.030, that of urine after incubation, 1.022. Difference, $8 \times 0.23 = 1.84\%$.

It is advisable to have two good urinometers, one to register from 1000 to 1025, a second to register from 1025 to 1050.

Benedict's New Method for Quantitative Determination of Sugar in Urine.

The solution for quantitative work has the following composition:

Copper sulphate (pure crystallized)	18.0 c.c.
Sodium carbonate—crystallized (100 grams of anhydrous salt will answer)	200.0 gm.
Sodium or potassium citrate	200.0 gm.
Potassium sulphocyanate	125.0 gm.
5 % potassium ferrocyanid solution	5.0 c.c.
Distilled water to make total volume of	1000.0 c.c.

With the aid of heat dissolve the carbonate, citrate and sulphocyanate in enough water to make about 800 c.c. of the mixture, and filter if necessary. Dissolve the copper sulphate separately in about 100 c.c. of water and pour the solution slowly into the other liquid, with constant stirring. Add the ferrocyanid solution, cool and dilute to exactly one liter. Of the various constituents, the copper salt only need be weighed with exactness. Twenty five c.c. of the reagent are reduced by 50 mg. of glucose.

Sugar estimations are conducted as follows: The urine, 10 c.c. of which should be diluted with water to 100 c.c. (unless the sugar content is believed to be low), is poured into a 50 c.c. burette up to the zero mark. Twenty-five c.c. of the reagent are measured with a pipette into a porcelain evaporating dish (25–30 cm. in diameter),

10 to 20 gm. of crystallized sodium carbonate (or one-half the weight of the anhydrous salt) are added, together with a small quantity of powdered pumice-stone or talcum, and the mixture heated to boiling over a free flame until the carbonate has entirely dissolved. The diluted urine is now run in from the burette, rather rapidly until a chalk white precipitate forms, and the blue color of the mixture begins to lessen perceptibly, after which the solution from the burette must be run in a few drops at a time, until the disappearance of the last trace of blue color, which marks the end point. The solution must be kept vigorously boiling throughout the entire titration. If the mixture becomes too concentrated during the process, water may be added from time to time to replace the volume lost by evaporation. The calculation of the percentage of sugar in the original sample of urine is very simple. The 25 c.c. of copper solution are reduced by exactly 50 mg. of glucose. Therefore the volume run out of the burette to effect the reduction contained 50 mg. of the sugar. When the urine is diluted 1:10, as in the usual titration of diabetic urines, the formula for calculating the per cent. of sugar is the following:

$\frac{0.050}{X}$ times 1000 equals per cent. in original sample, wherein X is the number of cubic centimeters of the diluted urine required to reduce 25 c.c. of the copper solution.

In the use of this method chloroform must not be present during the titration. If used as a preservative in the urine it may be removed by boiling a sample for a few minutes, and then diluting to its original volume.

This solution will keep indefinitely and it is claimed by Benedict, that comparison with the polariscope and by Allihn's gravimetric process will show it to be more accurate than any of the ordinarily used methods.

Approximate quantitative estimation with Fehling's solution. (One c.c. of Fehling's solution is reduced by 5 mg. glucose.)

Measure off 2 c.c. of Fehling's solution in a pipette and put in a test-tube or small beaker and dilute with 20 c.c. of water.

Bring the diluted Fehling's to boiling and drop in drop by drop the urine from a dropping-bottle for which the number of drops per c.c. has been noted. Estimating 20 drops to the c.c. if 2 drops of urine are required to reduce the copper it would show a sugar percentage of the urine of 10. Four drops 5%, 8 drops 2.5%, 16 drops 1.25%, 32 drops 0.6%, 64 drops 0.3%, 100 drops 0.2%.

The Pancreatic Reaction of Cammidge in the Urine.

Cammidge claims that there is a definite and important relationship between his pancreatic reaction in the urine and disease of the pancreas. The results of some workers go to support this view, particularly when considered in connection with the examination of the fæces for neutral fat.

The principle of the reaction depends upon the formation in the urine of a substance having the characters of an unfermentable pentose sugar after boiling with hydrochloric acid. It is not present in the original urine as such, and forms an osazone on treatment with phenylhydrazine, easily distinguished from the corresponding compound of glucose. As the presence of glucose would seriously interfere with the success of the reaction, all specimens of the urine examined must be care-

fully tested for glucose, and, if present, it must be removed by fermentation with yeast cake. Glucose is rarely present.

The technic of the reaction requires considerable time, but is easy of manipulation, and should be readily carried out in any hospital. The urine, if alkaline, must be made acid in reaction, and any albumin or sugar present must be removed and the urine made up to its original bulk with distilled water. To 40 c.c. of the clear filtered urine are added 2 c.c. of concentrated hydrochloric acid, and the mixture gently boiled for ten minutes in a small flask, using a funnel in the neck as a condenser. It is now cooled and distilled water added to again make up the contents to 40 c.c., owing to the loss by evaporation. Eight grams of lead carbonate are now slowly added to neutralize the excess of acid. After standing for a few minutes the flask is again thoroughly cooled and the contents filtered until perfectly clear. The filtrate is then well shaken with 8 grams of powdered tribasic lead acetate, and the resulting precipitate removed by filtration, which is repeated until perfectly clear.

The excess of lead in solution must now be removed by treating with 4 grams of powdered sodium sulphate; the mixture is heated to boiling, then thoroughly cooled and filtered. From the filtrate are measured 17 c.c.; this is transferred to a small flask with funnel condenser and there are added 2 grams of sodium acetate, 0.8 grams phenylhydrazine hydrochloride and 1 c.c. of 50% acetic acid. The mixture is then boiled gently for ten minutes, filtered into a test-tube with a mark showing 15 c.c., and made up, if necessary, to that point with hot distilled water. The filtrate is carefully stirred and left to stand over night.

The quantity and time of deposit of the crystals will depend upon the degree of extension of the inflammatory process in the pancreas. Thus, in well-marked cases, a light-yellow flocculent precipitate should appear in a few hours, but in less characteristic cases it may be necessary to leave the preparation over night before a deposit occurs. Under the microscope the precipitate is seen to consist of long, light-yellow, flexible, hair-like crystals of pentosazon, arranged in delicate sheaves.

Urinary Tests in Connection with Acidosis.

The determination of the ammonia quotient, which is the ratio of N eliminated as ammonia to total nitrogen elimination, has assumed great importance by reason of its connection with various forms of acid intoxication, as in diabetes, pernicious vomiting of pregnancy, and various hepatic diseases.

The degree of acidosis is better determined by the quantitative estimation of nitrogen elimination as ammonia than by estimating quantitatively the amount of diacetic and β-oxybutyric acid in the urine. Normally we have about 0.7 gram of ammonia eliminated daily. In acidosis this may rise to 5 or 10 grams and instead of being from 3 to 5% of the total N, it may amount to 30 to 50%.

Formalin Method for the Estimation of Ammonia.

Free ammonia reacts with formalin to form hexamethylenetetramine. If sodium hydrate is added to neutralized urine in the presence of formalin free ammonia is liberated and reacts with the formalin. So soon as all the ammonia has been liberated, the end reaction occurs.

Ronchese first utilized this principle and Mathison found that pot. oxalate made the end reaction sharper. Brown found that preliminary clearing with lead subacetate made the end reaction still sharper and removed certain nitrogenous substances which reacted with formalin making the result only about 5% higher than with Schaffer's method. The technic is as follows: About 60 c.c. of filtered urine are treated with 3 grams of basic lead acetate, well stirred, allowed to stand a few minutes and filtered. The filtrate is treated with 2 grams of neutral potassium oxalate well stirred and filtered; 10 c.c. of the clear filtrate are diluted to 50 c.c. with distilled water; a few drops of 1% phenolphthalein solution are added. The mixture will be slightly alkaline or acid. Five grams potassium oxalate are added and stirred. It is exactly neutralized with decinormal NaOH or H_2SO_4. Twenty c.c. of 20% commercial formalin, previously made neutral, are added, and the solution again titrated with decinormal NaOH to neutralization. Every c.c. of decinormal NaOH corresponds to 0.0017 gram NH_3. The quantity of ammonia is then calculated on the basis of the twenty-four-hour volume. Example: The 10 c.c. of urine required 4 c.c. N/10 NaOH to give a pink color. $4 \times 0.0017 = 0.0068$. Then 100 c.c. urine would contain 0.068 and 1000 c.c. (twenty-four-hour urine amount) 0.68 gram of ammonia.

Estimation of Total Nitrogen.

Principle.—The nitrogenous material of the urine is converted into ammonium sulphate on boiling with H_2SO_4. The ammonia is then estimated as described under estimation of ammonia by the formalin method.

Technic.—Solutions required:

1. Twenty per cent. commercial formalin previously made neutral with NaOH.
2. N/10 NaOH.
3. Forty per cent. NaOH.

Ten c.c. of filtered urine are pipetted into a Kjeldahl or Koch flask; 10 c.c. of concentrated H_2SO_4 and 10 grams K_2SO_4 are added. The mixture is heated over a free flame, gently at first to avoid foaming, and is finally brought to a boil, which is continued until the mixture is perfectly clear, usually requiring forty-five minutes to an hour. The contents are cooled and quantitatively transferred to a 200 c.c. volumetric flask and 1 c.c. of phenolphthalein solution added. The greater part of the acidity is now neutralized by adding about 30 c.c. of the 40% NaOH. It is cooled under a water tap and made up to the 200 c.c. mark; 10 c.c. are taken, diluted to 50 c.c. with distilled water and exactly neutralized with N/10 NaOH. Twenty c.c. of the formalin solution are now added and the titration again performed. The pink end reaction is beautifully clear and sharp. The second reading multiplied by the factor 0.0014 gives the amount of nitrogen in grams in 10 c.c. of the fluid. It is then computed for the twenty-four-hour volume as for N, eliminated as ammonia. Example: It required 5 c.c. N/10 NaOH—$5 \times 0.0014 = 0.007$. As original 10 c.c. were diluted to 200, the 10 c.c. taken for titration would only be 1/20; hence $0.007 \times 20 = 0.14$ gram for 10 c.c or 1.4 for 100 c.c. or 14 grams for 1000 c.c.

The amount of urea, which represents from 85 to 90% of the total nitrogen, is usually determined instead of the total N. The hypobromite and hypochlorite methods are, however, lacking in accuracy, and more exact methods of urea estimation are more time-consuming than the one just given for total N.

Probably the most convenient test for urea is the hypobromite method, using the Doremus ureometer with a side tube connected to the closed arm of the fermentation tube by a glass stop cock.

The reagent is prepared by taking 70 c.c. of a 30% stock solution of NaOH, diluting it with 180 c.c. water and then adding 5 c.c. of bromine, stirring until the bromine is dissolved. This solution if stored in a cool dark place will keep about one week.

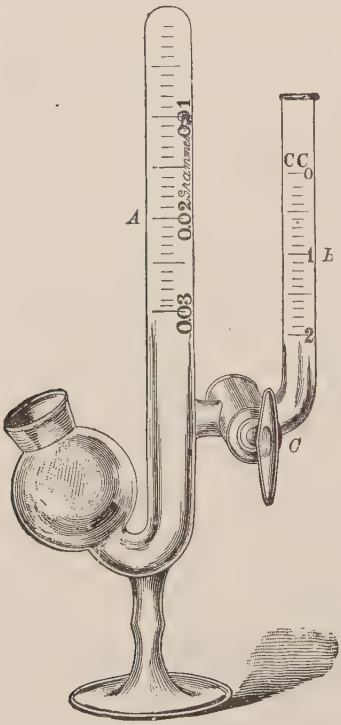


FIG. 106.—Doremus-Hinds Ureometer.

The urine to be tested must be free from sugar and albumin and contain less than 1% of urea. Ordinarily the urine must be diluted two to four times to obtain a specimen containing less than 1%. In using this improved Doremus ureometer the closed portion of the U tube is filled with the hypobromite solution, and the urine introduced by allowing it to run in from the side tube by opening the glass cock arranged for that purpose. After the gas has risen and the instrument has stood for a short time the readings may be made in grams to the liter, or in percentage.

This urea determination is only a rough clinical one.

Gerhardt's Test for Diacetic Acid.

Add a few drops of ferric chloride solution to 10 to 50 c.c. of urine so long as a precipitate continues to form. Then filter and to the filtrate add more ferric chloride solution. A bordeaux red color shows diacetic acid. The test is sensitive. As a control to show that the color is not due to drug elimination (antipyrine, salicylates, etc.) boil a specimen which gave the test for three to five minutes. If the color was due to drugs it will be obtained with a boiled sample while such treatment drives off the diacetic acid. In Hurtley's test add 2.5 c.c. HCl and 1 c.c. of 1% sol. of sod. nitrate to 10 c.c. urine. Shake and allow to stand 2 minutes. Now add 15 c.c. strong ammonia followed by 5 c.c. of 10% sol. ferrous sulphate. The slow production of a violet colour shows positive test (2 hours). Shows 1 part aceto-acetic acid in 50,000.

If the urine shows a well-marked Gerhardt reaction it is well to test for β-oxybutyric acid.

The following modification of Lange's test by Hart is a satisfactory one. The principle involved is the removal of acetone and diacetic acid by heat, then oxidizing β-oxybutyric acid to acetone with hydrogen peroxide and then testing for acetone.

Method: Take 20 c.c. of urine, dilute with an equal amount of water and add a few drops of acetic acid. Next boil in a beaker until the original amount of diluted urine is reduced to 10 c.c. (originally 40 c.c.). Dilute this evaporated urine with an equal amount of water, giving us 20 c.c. In each of two test-tubes put 10 c.c. of this 20 c.c. To one tube add 1 c.c. of hydrogen peroxide and warm gently, without boiling, for one minute; then cool. The other tube is left untreated. Next, to each test-tube add 10 drops of glacial acetic acid and 5 to 10 drops of a freshly prepared sodium nitroprusside solution and mix. Next carefully overlay each tube with about 2 c.c. of concentrated ammonia. If β-oxybutyric acid were present in the tube treated with the hydrogen peroxide and thereby oxidized to acetone a violet-red ring will develop at the point of contact while in the untreated tube there will be no such color ring.

A yellowish-brown ring from the presence of creatinin may show in the untreated tube. It is well to allow the tubes to stand for three to four hours before finally reporting the absence of β-oxybutyric acid. It will probably show 0.2%.

Acetone.—To one-sixth of a test-tube of urine add a crystal of sodium nitroprusside. Make strongly alkaline with NaOH. Shake. The addition of a few drops of glacial acetic gives a purple color to the foam, if acetone is present.

Diazo Reaction.—To 5 c.c. sulphanilic acid solution (sulphanilic ac. 1 pt., HCl 50 pts., aq. 1000 pts.) add two drops of a 0.5% solution of sodium nitrite. Add an equal quantity (5 c.c.) of urine. Shake and add quickly 2 or 3 c.c. of ammonium hydrate. A carmine color, especially in the foam, shows a diazo reaction. If the reaction is positive, and the mixture is allowed to stand for 24 hours, a precipitate forms, the upper margin of which exhibits a green, greenish-black or violet zone.

Indican.—Take 10 c.c. urine and treat it with 1 c.c. of sol. of lead subacetate. Filter. Of this filtrate take 6 c.c. and treat with an equal amount of Obermayer's reagent; allow to stand for 5 minutes then shake gently with 2 c.c. of chloroform. Obermayer's reagent is strong HCl containing 2 parts of ferric chloride to the liter —0.1 gram to 50 c.c. of HCl.

A more exact method is to pour off the supernatant acid urine. Wash the chloroform with water, then pour off as much of the supernatant water as possible and add 10 c.c. of alcohol. A clear blue fluid results.

Urobilin.—Urobilin appears in considerable quantity in urine when there is much destruction of red cells, as in pernicious anæmia, internal hæmorrhage, and in malaria cachexia. The best test is that of Schlesinger. To the unfiltered urine add an equal amount of a saturated solution of zinc acetate in absolute alcohol. Shake, add a few drops of Lugol's solution and filter. Fluorescence in the filtrate shows the presence of urobilin. The degree of blood destruction is indicated by the intensity of the fluorescence.

Bile Pigments.—A satisfactory test is that of Rosin (Trousseau). Overlay 10 c.c. urine with about 5 c.c. of dilute tincture of iodine (1 to 10 of 95% alcohol). An emerald green ring at the point of contact shows the presence of bile coloring matter.

Phenolsulphonephthalein Test for Renal Efficiency.

Geraghty has recently stated that in 35 cases where an autopsy made it possible to verify the accuracy of this test that the lesions as revealed at autopsy corre-

sponded closely with the results of the test. Again in 30 nephrectomies the conditions found were in accordance with the results of the test. The general opinion of those who have used the test is that it is more reliable than cryoscopy and far easier of application. The technic is as follows: One c.c. of the phthalein solution containing 6 mg. is injected intramuscularly or subcutaneously. The drug can be bought in ampules ready for use. About twenty minutes before injecting the drug the patient is given from 200 to 400 c.c. of water to drink. After the injection the bladder is emptied with a catheter and the time is accurately noted when the urine which subsequent to the emptying of the bladder and being allowed to drop into a test-tube containing one drop of a 25% sodium hydrate solution first shows a pinkish tinge. This is recorded as the time of appearance of the drug in the urine and normally is about 10 minutes. The catheter is then withdrawn and the urine that is passed in the first hour collected and subsequently that passed in the second hour. To each hour's specimen sufficient 25% sodium hydrate is added to give a purple-red color and the entire amount is then poured into a liter flask and made up to 1000 c.c. A similar treatment is employed for the urine of the second hour. The amount of drug eliminated in each hour is then determined by a colorimeter.

Cabot has proposed the use of a series of ten test-tubes containing solutions of the drug representing from 5% to 50% of the drug dose, each tube containing 5% more than the preceding one. These comparison solutions may be made up with the patient's urine obtained at the time of emptying the bladder so that the confusion which may obtain when water is used is avoided. It has recently been proposed to make the standards with water and use a piece of yellow glass for matching. The urine to be tested made up to 1000 c.c. as previously described is then poured into a test-tube of similar size and matched.

In normal cases Cabot got 46% of the drug eliminated in the first hour, the average for the second hour being 17%. The quantity of urine secreted in either hour has no relation to the test, which is the percentage of drug eliminated. In cases with serious kidney disease the amount of drug eliminated in the first hour may range from 5 to 12%.

When the question of the kidney involved arises, the urine must be taken by ureteral catheterization or by a separator.

F—CHEMICAL EXAMINATION OF GASTRIC CONTENTS.

The test breakfast ordinarily used is that of Ewald (one shredded wheat biscuit or two small pieces of toast with 400 c.c. of water is what is usually given). This Ewald breakfast is a low-grade stimulant to acid production. It is given in the morning on an empty stomach. If at supper, the night before, the patient partake of raspberry jam the finding of the characteristic seeds in the stomach contents the next morning would be evidence of lack of motor activity. The Fischer meal which contains a 4-ounce Hamburg steak in addition to the water and toast of the Ewald is withdrawn after three hours.

The stomach tube is more easily passed if it be thoroughly chilled in ice water without the use of any lubricant.

The stomach tube should be passed one hour after the Ewald breakfast and if more than 50 c.c. of fluid be obtained it indicates stasis or hypersecretion.

Filter the gastric contents and test first for free HCl. The most reliable and sensitive test is that of Gunsberg. The reagent, which should be freshly prepared, consists of phloroglucin 3 grams, vanillin 1 gram, and absolute alcohol 30 c.c. By mixing 2 drops of gastric juice and an equal quantity of Gunsberg reagent in a small porcelain dish and carefully heating above a flame we obtain a carmine red color if free HCl be present. A water bath is preferable.

For lactic acid a modification of Strauss' method is quite satisfactory. Shake, in a test-tube, 5 c.c. of gastric contents with 20 c.c. of ether, allow to settle and pour off 5 c.c. of the supernatant ether into another test-tube. To this ether add 20 c.c. of water and 2 drops of a 1 to 9 solution of ferric chloride and shake well. The presence of 1% of lactic acid will give an intense greenish color.

Having determined the presence or absence of free hydrochloric or lactic acid, we should make a quantitative test of the various factors producing the acidity of gastric juice (a modified Töpfer test). These are: 1. Free HCl. 2. Combined HCl. 3. Acid salts, and 4. Total acidity.

To 10 c.c. of filtered gastric contents, in a beaker, add 3 drops of dimethyl-amido-azo-benzol solution (a 1/2% solution in 95% alcohol). In the presence of free HCl the fluid becomes a rich carmine pink.

After reading the burette run in N/10 NaOH solution until the pink color is discharged and a light yellow color is obtained. This reading multiplied by 10 gives the amount of free HCl in degrees, a degree corresponding to 1 c.c. N/10 NaOH. Next add 6 drops of a 1/2% alcoholic solution of phenolphthalein to the light yellow fluid in the beaker. Again titrating the same preparations we add N/10 NaOH until a faint but distinct pink color is produced. The number of c.c. added for the free HCl plus the number to give the pink color when multiplied by 10 gives the total acidity in degrees. (For example: 2.5 c.c. N/10 NaOH used to obtain yellow color—$2.5 \times 10 = 25$ or acidity due to free HCl. After adding the phenolphthalein, 4 c.c. N/10 NaOH required to produce pink color—$4 + 2.5 \times 10 = 65$ or total acidity in terms of acidity. This means that it would require 65 c.c. N/10 NaOH to neutralize 100 c.c. of gastric juice. A total acidity of 60 is about normal. To obtain percentage in HCl multiply by 0.00365; thus. $65 \times 0.00365 = 0.23$ HCl.)

Having determined the total acidity add 3 c.c. of 10% neutral calcium chloride solution to the gastric contents already in the beaker. As a result of the formation of acid calcium phosphate the pink color is discharged. Again add N/10 NaOH from the burette until the pink color is restored. The number of c.c. used gives the amount of acid salts present.

From the figures for the total acidity subtract the sum of that for free HCl and for acid salts and the remainder will give the acidity due to combined HCl.

G—CHEMICAL TESTS OF FÆCES.

To test for acidity Kaplan rubs up 5 grams fæces in 30 c.c. distilled water. Put 2 c.c. of the emulsion in a test-tube and add a few drops of phenolphthalein solution. Titrate with N/10 NaOH to a pink. A normal stool from a Schmidt test-diet requires about 1.5 c.c. N/10 NaOH. After fermentation the stool may be quite acid or more alkaline than before the fermentation test.

Test for Pancreatic Ferments.—To obtain a stool for ferment examination, calomel 2 to 3 grains, or phenolphthalein 5 grains is to be preferred to salts. The Fuld-Gross-Goldschmidt test uses for trypsin testing a solution of casein, 1 gram, sodium carbonate, 1 gram chloroform 1 c.c. to 1 liter of water. If the stool is not very liquid 5 grams of fæces are rubbed up with 20 c.c. salt solution and filtered. Dilutions of 1 to 10, 1 to 100 and 1 to 1000 are made and 0.5 and 1 c.c. of these dilutions added to 6 test-tubes each containing 5 c.c. of the casein solution. The tubes are incubated for 24 hours at 38° C. and completion of the digestion tested by adding 5% acetic acid which should not cause a precipitate in tubes in which digestion is complete.

The estimation is made by units, one unit being the digestive power of one c.c. of fæces filtrate to digest 1 c.c. of casein solution. If 1 c.c. of the 1 to 1000 fæces dilution digested 5 c.c. of casein solution it would represent 5000 units. If 1 c.c. of 1 to 10 dilution it would be 50. As there are 5 c.c. of the casein solution we multiply the dilution of fæces by 5 for 1 c.c. or by 10 if we had only 0.5 c.c. of fæces dilution in the tube tested.

For amylopsin a similar technic is followed using a 1% solution of soluble starch instead of the 0.1%. casein solution. The end reaction is tested by adding 1 drop of N/10 iodine solution to each of the starch tubes and fæces dilution after 24 hours of incubation. The absence of a blue color shows completion of starch digestion.

The normal ferment content of the fæces rarely falls below 200 units and may be as high as 10,000. Cases showing a ferment value of only 25 to 50 units are very suspicious as regards pancreatic disease.

H—DISINFECTANTS AND INSECTICIDES.

By disinfection is meant the destruction of injurious bacteria.

Sterilization is where all living things are destroyed.

Germicides are substances which kill bacteria while antiseptics are those which are inimical to the growth of bacteria.

Formalin is antiseptic in 1–50,000 dilution but germicidal only in 1–20.

Deodorants may or may not be antiseptic or germicidal. An insecticide may or may not be a germicide and *vice versa*.

In disinfection we must consider

(1) Strength of solution.

(2) Time of application.

(3) Nature of medium in which disinfectant acts.

By Coefficient of Inhibition we mean time and concentration necessary to prevent development of bacteria.

By Inferior Lethal Coefficient we mean time and concentration necessary to kill nonspore-bearing bacteria.

By Superior Lethal Coefficient we mean time and concentration necessary to kill spore-bearing bacteria.

Disinfectants may be (A) physical (B) gaseous (C) chemical.

(A) **Of the physical disinfectants** we have

(1) Sunlight. The red and yellow rays practically inert. The violet and

ultra violet most active. Direct sunlight kills plague bacilli in less than one hour —typhoid bacilli in six.

(2) Burning. Very efficient but expensive.

(3) Boiling. Especially in carbonate of soda solution for about one hour is a very efficient disinfectant. Nonspore-bearing bacteria are killed almost instantly by a boiling temperature. One must remember that the boiling temperature is lower at mountainous elevations.

(4) Steam. Extremely efficient. The condensation of the steam on the object to be sterilized gives off latent heat and produces a vacuum.

(B) **Of the gaseous disinfectants** we have the very efficient germicide formaldehyde gas and the weakly germicidal, but potent insecticide, sulphur dioxide.

Formaldehyde gas is practically valueless as an insecticide.

Bromine, chlorine and hydrocyanic acid gas have a certain degree of efficiency but are not of practical application. Hydrocyanic acid gas is especially dangerous on account of its extreme toxicity.

(1) **Formalin.**—This is a 40 % solution of formaldehyde gas, but is as a rule of less strength from evaporation or otherwise. Formaldehyde is efficient as a surface disinfectant when the temperature is above 50° F. and the air contains at least 60 % of moisture. It is not efficient in cold dry rooms. Owing to its lack of penetrating power it is not efficient for the disinfection of mattresses, or similar articles. To prepare a room for disinfection we must measure the cubic space to ascertain the necessary amount of formalin to use and stuff up or better paste up with newspaper all cracks and openings.

In the production of formaldehyde gas the more expensive autoclaves and lamps have largely been replaced by the simple formalin permanganate method. In this one pours 500 c.c. of formalin on 250 grams of potassium permanganate for each 1000 cubic feet with six to twelve hours' exposure.

In employing this method, take a pan partly filled with water. Place in this a second metal or glass receptacle containing the permanganate. Then pour the formalin on the permanganate crystals. The gas is generated in great amount in a few seconds. The receptacle containing the permanganate and formalin should be large enough to contain ten times the volume of formalin, as there is a tendency for the mixture to foam over the sides of the dish.

Another practical method is the **formalin-sheet-spraying** one. The formalin (40%) should be sprayed on sheets suspended in the room in such a manner that the solution remains in small drops on the sheet. Spray not less than 10 ounces of formalin (40%) for each 1000 cubic feet. Used in this way a sheet will hold about 5 ounces without dripping or the drops running together. The room must be very tightly sealed in disinfecting with this process and kept closed not less than twelve hours. The method is limited to rooms or apartments not exceeding 2000 cubic feet. The formalin may also be sprayed upon the walls, floors, and objects in the rooms.

Paraform Lamps.—For single rooms the use of the paraform lamp is quite convenient. Special lamps can be obtained to burn the paraform tablets or a pint tincup will suffice for the heating of 1 ounce of paraform. The lamp or alcohol flame under the receptacle must not be high enough to ignite the paraform which burns readily and in so doing does not give off formaldehyde gas. One ounce of

paraform is sufficient for a space of 500 cubic feet. One can dissolve 2 ounces of paraform in 8 ounces of boiling water and then pour this over 4 ounces of potassium permanganate in a two gallon pail.

N. Y. Health Department Method.—After a prolonged series of tests the N. Y. Department of Health gave preference to the following formula.

Paraformaldehyde 30 grams, potassium permanganate 75 grams, water 90 grams. The chemicals are mixed in a deep quart pan and the water is added and the mixture stirred. The evolution of gas is slow in starting but is complete in five to ten minutes.

It was found that 87% of the gas was evolved and the quantities given above suffice to disinfect 1000 cubic feet in four hours. It is well to put the small pan containing the chemicals in a larger one to prevent danger of fire and soiling of the floor by the frothing of the mixture.

Sulphur Dioxide.—Sulphur dioxide is fairly efficient, but requires the presence of moisture. It is only a surface disinfectant and is lacking in penetrating properties. An atmosphere containing 4.5%. can be obtained by burning 5 pounds of sulphur per 1000 cubic feet of space. This amount requires the evaporation or volatilization of about 1 pint of water. Under these conditions the time of exposure should be not less than twenty-four hours for bacterial infections. A shorter time will suffice for fumigation necessary to kill mosquitoes and other vermin. Dry sulphur dioxide produced by burning 2 pounds of sulphur for each 1000 cubic feet of space will answer for this purpose. An exposure of from two to three hours is sufficient.

The sulphur may be burned in shallow iron pots (Dutch ovens), containing not more than 30 pounds of sulphur for each pot, and the pots should stand in vessels of water. The sulphur pots should be elevated from the bottom of the compartment to be disinfected in order to obtain the maximum possible percentage of combustion of sulphur. The sulphur should be in a state of fine division, and ignition is best accomplished with alcohol (special care being taken with this method to prevent damage to cargo or vessel by fire), or the sulphur may be burned in a special furnace, the sulphur dioxide being distributed by a power fan. This method is peculiarly applicable to cargo vessels.

Liquefied sulphur dioxide may be used for disinfection in place of sulphur dioxide generated as above, it being borne in mind that this process will require 2 pounds of the liquefied gas for each pound of sulphur, as indicated in the above paragraphs.

Sulphur dioxide is especially applicable to the holds of vessels or to apartments that may be tightly closed and that do not contain objects that would be injured by gas. Sulphur dioxide bleaches fabrics or materials dyed with vegetable or aniline dyes. It destroys linen or cotton goods by rotting the fiber through the agency of the acids formed. It injures most metals. It is promptly destructive of all forms of animal life. This property renders it a valuable agent for the extermination of rats, insects, and other vermin. Sulphur dioxide is a germicide only in the presence of moisture, and even then will not kill spore-bearing organisms. If clothing is washed immediately after sulphur disinfection the rotting effect will be greatly lessened. If used in spaces containing machinery all metal parts should be coated with vaseline.

Chemical Solutions.

Bichloride of mercury is usually sold in the form of antiseptic tablets. As a disinfectant for the infectious diseases it is usually used in a strength of 1–1000. The solution should be made in a wooden or earthenware vessel. As bichloride forms inert albuminates it should not be used in the disinfection of sputum, fæces or any albuminous excreta. It must be remembered that bichloride is a mordant so that any stains in soiled clothing will remain permanent. For disinfection of clothing the material should be left in 1–1000 bichloride for one hour. Dishes for food should never be disinfected in bichloride on account of the danger from poisoning. Floors and walls may be disinfected with 1–1000 bichloride applied with a mop. Allow the solution to dry on the floor or walls.

Formalin.—A 5% solution of commercial formalin in water (50 c.c. formalin 950 c.c. water) makes a satisfactory disinfectant for soiled clothing. It is also valuable for albuminous material. The disinfectant must act in a strength of 5% so that if one pint of fæces is to be disinfected we should add one pint of a 10% formalin solution and allow it to act for one hour.

Carbolic Acid.—It is soluble in water to the extent of about 5% and in such strength it is an efficient disinfectant. The solution should be made with hot water.

In standardizing disinfectants carbolic acid is used as the standard. It however is expensive and there is often difficulty in making up satisfactory solutions. More efficient and more convenient is the **Liquor cresolis comp. U. S. P.** This may be prepared by mixing up equal parts of cresol and soft soap as noted on page 12. This has a value according to tests made in the Hygenic Laboratory of 3, making it in tests without organic matter three times as efficient as carbolic acid. Under similar conditions lysol had a value of 2.12 creolin 3.25 and trikresol of 2.62.

Equal parts of a 5% solution of Liq. Cresol. Comp. and the fæces, urine or sputum to be disinfected is satisfactory for disinfection provided the mixture is allowed to stand for one hour. Liq. Cresol. Comp. (5%) is an excellent disinfectant for contaminated bedclothing, etc. It is also most suitable for the disinfection of floors and walls.

Lime.—It must be remembered that air-slaked lime is inert as a disinfectant. For disinfecting fæces freshly prepared milk of lime is excellent. It is made by mixing unslaked lime with four times its volume of water. An equal quantity should be added to the fæces to be disinfected.

Chlorinated Lime.—This can be purchased in air-tight containers and when the package is opened it should give off a powerful odor of chlorine.

For a working disinfectant solution add 1 pound to 4 gallons of water. This is satisfactory for mopping floors and for disinfecting fæces, sputum and urine, equal parts of the excreta and disinfecting solution being mixed and allowed to stand for one hour. For disinfection of drinking water one teaspoonful of chlorinated lime to 1 pint of water makes a stock disinfectant. For use one teaspoonful of this stock solution is added to 2 gallons of the drinking water to be disinfected. Let stand at least 1/2 hour.

Insecticides.

The following notes are taken chiefly from the U. S. P. H. Service directions.

Sulphur dioxide—obtained as described above—destroys all animal life.

In the case of vessels, when treated for yellow fever infection, the process shall be a simultaneous fumigation with sulphur dioxide, 2 % volume gas, and two hours' exposure, in order to insure the destruction of mosquitoes.

In the case of vessels when treated for plague the process with sulphur dioxide shall be as follows:

Without cargo: The simultaneous fumigation with sulphur dioxide gas not less than 2% for six hours' exposure.

With cargo: Fumigation with sulphur dioxide gas, 4 %, six to twelve hours' exposure, according to stowing.

Infected vessels may require partial or complete discharge of cargo, and fractional fumigation for efficient deratization.

Pyrethrum. The fumes of burning pyrethrum may be used to destroy mosquitoes in places where there are articles liable to be injured by the use of sulphur.

Four pounds per 1000 cubic feet space for two hours' exposure will kill, all or practically all of the mosquitoes but precautions should be taken to sweep up and destroy any that may have escaped.

Pyrethrum stains walls, paper, etc.

The oxides of carbon, as used at Hamburg, are efficient to destroy rats but do not kill fleas or other insects. They are obtained by burning carbon, coke, or charcoal, in special apparatus, and the gas as produced consists of about 5 % carbon monoxide, 18 % carbon dioxide, and 77 % nitrogen.

Twenty kilos of carbon, coke, or charcoal are used for every 1000 meters of space. The gas is allowed to remain in the ship for two hours and from seven to eight hours are allowed for it to leave it. This is about equivalent to 1 1/3 pounds of carbon (coke) to 1000 cubic feet of air space. As this gas is very fatal to man and gives no warning of its presence, being odorless, a small amount of sulphur dioxide should be added to give warning of its presence. As it does not kill fleas it cannot be depended on for complete work, where there is evidence of plague among rats on the vessel, as the infected fleas would infect the rats coming aboard after the deratization.

The articles named as disinfectants which can obviously destroy animal life can be used for that purpose when applicable, as steam for bedding, fabrics, etc. Formaldehyde is not applicable for this purpose.

For fleas the best insecticides are (1) crude petroleum (fuel oil) which is at times called Pesterine, (2) an emulsion of kerosene oil made as follows: kerosene 20 parts, soft soap 1 part and water 5 parts. The soap is dissolved in the water by aid of heat and the kerosene oil gradually stirred into the hot mixture.

For cockroaches there is nothing so good as sodium fluoride. By sprinkling the powder about the haunts of the cockroaches they are gotten rid of in a few days.

For exterminating rats and in this way secondarily the rat-fleas besides the ordinary poisons such as As., P., etc. Rucker has recommended a poison composed of plaster of Paris, 6 parts, pulverized sugar 1 part and flour 2 parts. This mixture should be exposed in a dry place in open dishes. To attract the rats the edge of the dish may be smeared with the oil in which sardines have been packed.

Wise and Minett report good results from the use of crude carbolic acid as a larvicide for mosquitoes. They added about 1 teaspoonful for each 2 cubic feet of water in the pool. Of course the ordinary method for destroying mosquito larvæ is by covering the surface of the water in the cistern or pool with a layer of petroleum.

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Equivalent Fahrenheit and Centigrade tables for the temperatures in common use in laboratories:

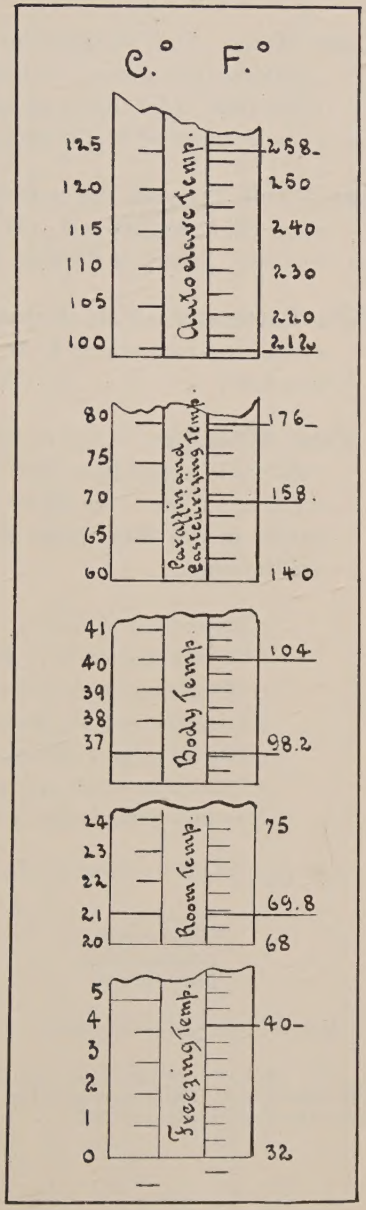
1. Those for sterilization of dressings and media. Also for certain disinfection of spore-bearing bacterial contamination (autoclave temperatures).

2. Those for pasteurization and sterilization of bacterial vaccines. Also for paraffin bath (pasteurizing temperatures).

3. Those for growing important pathogenic organisms (body temperatures).

4. Those for culturing gelatin (melting point, 25° C.) as in water work (room temperatures).

5. Those employed in preserving biological products and post-mortem material; also in centrifuging experiments to separate complement and amboceptor (freezing temperatures).



UNITS IN COMMON USE IN LABORATORIES.

Cubic Meter.—Unit of space for the number of organisms in air. It contains 1000 liters. It is equal to 1.308 cubic yards or 35.316 cubic feet. One thousand cubic feet, the unit of space in disinfection, is equal to 28.3+ cubic meters.

Liter.—Unit of space for normal volumetric solutions. It contains 1000 cubic centimeters. It is equal to 1.0567 quarts or 33.8+ ounces. A liter of distilled water weighs 1 kilogram.

Cubic Centimeter.—Unit of space for organisms in water, milk, vaccines, etc., 1 c.c. = 0.27 fl. dr. There are, approximately, 16 drops in 1 c.c.

Cubic Millimeter.—Unit of space for blood-cells. There are 1000 cubic millimeters in 1 cubic centimeter and 1 million cubic millimeters in 1 liter. In water analysis, as there are 1 million milligrams in one liter, parts in the million and milligrams per liter are the same.

1 Meter = 39.37 inches.
1 Centimeter = 0.3937 inch. Approximately, 2/5 inch.
1 Millimeter = 0.0393 inch. Approximately, 1/25 inch.
1 Kilogram = 2.2+ pounds av.
1 Gram = 15.432 grains.
1 Milligram = 0.0154 grain. Approximately, 1/64 grain.
A pound avoirdupois is equal to 453.59 gm.

One hundred cubic centimeters of a saturated solution contains:

	Water	Alcohol
Methylene blue,	6.68	0.66 gram.
Gentian violet,	1.75	4.42 grams.
Basic fuchsin,	0.66	2.92 grams.

Key to Table on opposite page.

− = negative + = positive, O = no change, A = acid, Alk. = alkaline, G = gas; Fl = fluorescence, Pep = peptonization, 1 = litmus Russell's medium not reduced, 2 = reduced, 3 = action variable.

Important non-spore bearing Gram negative intestinal bacilli.	Capsules	Motility	Gelatine	Milk (coagulation)	Litmus Milk			Glucose	Maltose	Lactose	Mannite.	Saccharose	Glucose neutral red bouillon	Russell's Medium		Indol.	Voges-Proskauer	Remarks
					1st day	3d day	12th day							Butt	Slant			
1 B. fæcalis alkaligines.	–	+	–	–	Alk	Alk	Alk	O	...	O	O	O	O	O	O	–	–	Found in fæces and sewage-contaminated water. Differs from B. typhos. by marked alkali production.
2 B. typhosus.	–	+	–	–	A	A	A Alk	A	...	O	A	O	O	A	Alk	–	–	Blood cultures first week—agglutination afterward.
3 B. dysenteriæ (Shiga-Kruse).	–	–	–	–	A	Alk	Alk	A	O	O	O	O	O	A	Alk	–	–	Nonacid strain, highly toxic.
4 B. dysenteriæ (Flexner-Strong).	–	–	–	–	A	Alk	Alk	A	A	O	A	O	O	A	Alk	–	–	Acid mannite strain, moderate toxicity
5 B. dysenteriæ "Y."	–	–	–	–	A	Alk	Alk	A	O	O	A	O	O	A	Alk	+ –	–	Much like Flexner strain. No acid in maltose.
6 B. Morgan No. 1.	–	+	–	–	O	O	Alk	A G	...	O	O	O	G	.....	.....	+	–	Found in summer diarrhœa of children
7 B. paratyphosus A.	–	+	–	–	A	A	A Alk	A G	...	O	A G	O	G	A G	Alk	–	–	Little gas. No fluorescence n. red. Litmus milk acid in third day. 1.
8 B. paratyphosus B.	–	+	–	–	A	Alk	Alk	A G	...	O	A G	O	G Fl	A G	Alk	–	–	Much gas. Marked reduction n. red with yellow fluorescence. Litmus milk alkaline third day. 2.
9 B. enteritidis. (Gaertner.)	–	+	–	–	A	Alk	Alk	A G	...	O	A G	O	G Fl	A G	Alk	–	–	B. choleræ suis, B. icteroides, B. of Danysz virus and B. paratyphoid B. closely related (Gaertner group). 2.
10 B. coli.	–	+	–	+	A	A	A	A G	...	A G	A G	O	G Fl	A G	A	+	–	There is also a B. coli anærogenes which is like B. coli but does not form gas
11 B. lactis ærogenes.	+	–	–	+	A	A	A	A G	...	A G	A G	A G	G Fl	A G	A	– +	+ +	Very nearly related to Friedlander's bacillus as well as to B. coli. 3.
12 B. cloacæ.	–	+	+	+	O	A	Pep.	A G	...	A G	A G	A G	G Fl	A G	A	+	+	Differs from B. coli in liquefaction of gelatin and shows slow production of gas in lactose.
13 B. proteus vulgaris.	–	+	+	+	O	Alk	Pep.	A G	...	O	.....	.....	G	A G	Alk	–	..	Three types—Proteus vulgaris rapid gelatin liq.; P. mirabilis, slow gelatin liq.; P. zenkeri, no gelatin liq. Spreading growths characteristic. 2.